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RESEARCH ARTICLE

ULTRAVIOLET-VISIBLE DETERMINATION OF ACTIVE CHEMICAL COMPOUNDS, ANTIRADICAL POTENTIALAND IN VITRO TOXICITY OF AGERATUM CONYZOIDES LINN (ASTERACEAE) USED BY GUINEAN HEALERS FORWOUND TREATMENT

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ABSTRACT

Wounds are classified as neglected tropical diseases. Herbal medicines are widely used and play a significant role in the alternative management of infectious diseases, including chronic wounds, ulcers and injuries. The overall aim of this study is to evaluate the antioxidant activity and major compounds of the hydroethanolic extract of the leafy stems of Ageratum conizoides, following in vitro testing for cytotoxic activity. Phytochemical analysis was carried out using the standard differential reaction method. The major compounds with proven pharmacological properties were identified using UV-visible absorption spectrophotometry at characteristic maximum absorption wavelengths. Antioxidant activity was determined using the DPPH radical scavenging assay. Cytotoxic activity was determined using the Artemia salina cell mortality rate assay. The hydroethanolic crude extract contains higher amounts of saponins (957.2±2.42 mgEqAG/g), condensed tannins (491.02±1.03 mgEqCAT/g) and alkaloids (411.15±10.66 mgEqAG/g) than phenolic compounds (389.70 ± 0.36 mgEqAG/g), flavonoids (380.57 ± 2.15 mgEqAG/g) and triterpenes (310.50 ± 0.26 mgEqAG/g). The hydroethanolic extract significantly reduced the DPPH radical with a half-maximal inhibitory concentration (IC_{50}) of 6.98 mg/mL. No cellular safety was observed for the extract with an LC50 of 1.62 mg/ml.

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INTRODUCTION

Ageratum conyzoides Linn. is a herbaceous plant used for traditional medicinal and agricultural purposes in several countries around the world. It is one of the plants renowned in folk medicine for its antimalarial properties. The plant has been known since ancient times for its healing properties and has been used to treat various conditions, such as burns and wounds, infectious diseases, arthritis and fever (Booker *et al*., 2012). More specifically, it has been shown to exert hepatoprotective and radioprotective effects (Kanko *et al*., 2004). A. conyzoides is rich in flavonoids (Borris, 1996), some of which have been identified in the whole plant. Chronic inflammatory diseases remain one of the major health problems. Inflammation involves a complex series of enzymatic activations, tissue degradation and repair (Ndiaye *et al*., 2005). Medicinal plants could pave the way for the discovery of new anti-inflammatory drugs with fewer adverse effects (Bruneton, 2009). Nowadays, according to the WHO, around 80 per cent of the world's population continues to rely primarily on herbal remedies (WHO, 2010; Houngbeme *et al*., 2026). In Guinea, and particularly in the Kindia prefecture, which is characterised by a mix of Soussou and Fulani populations, the leafy stems of the plant are frequently used to treat wounds and chronic sores (Barry *et al*., 2006). This study focuses on identifying and determining the content of chemical groups recognised for their wound-healing properties, as well as the species' ability to combat the agents of oxidative stress responsible for several conditions associated with pain and wound inflammation.

MATERIAL AND METHODS

Material: Leafy stems of *Ageratum conyzoides* were collected in the Kindia region, specifically in Foulayah, in the morning

etween 9.00 am and 11.00 am. They were sent to the organic chemistry laboratory, where they were sorted and left to dry on laboratory benches at a constant temperature of 18°C for 12 days. Following this stage, the dried material was ground using a mill, and the powder was separated using an aluminium sieve with a mesh size of 710 µm. Artemia salina Leach larvae, cultured in vitro, were used as test organisms for the toxicity test. Figure 1 shows the whole plant of the species under study.

Fig 1 : ***Ageratum conizoides***

Methods

Preparation of the crude extract: The hydroethanolic extract was prepared using the maceration method with a binary solvent mixture, as described in the studies by Houngbèmè *et al*, 2025 and Houngbèmè *et al*, 2026. 100 g of pulverised plant material was mixed with 1 L of a mixture of distilled water and 96° ethanol in a ratio of 4–6 (v/v), then left to macerate continuously for 72 hours using a reciprocating magnetic stirrer. After filtering the macerate through cotton wool, the liquid was poured into a flask and concentrated in a rotary evaporator at 50°C, then dried in an oven at a constant temperature of 50°C to obtain the final dry extract.

Tests for identifying chemical groups: The various classes of chemical compounds (alkaloids, polyphenols, terpenes and steroids) contained in the crude hydroethanolic extract were identified using the colouring and precipitation method described in the works of Koudjina *et al*., 2023; Deguenon *et al*., 2023; Houngbeme *et al*., 2026. Mayer's and Dragendorff's tests for alkaloids, Fehling's test for free reducing sugars and glycosides, Liebermann-Burchard's test for triterpenoids and steroids, the frothy test for saponins, Shinoda's test for flavonoids, the ferric chloride test for tannins, Guignard's test for free cyanogenic derivatives and Borntrager's test for free anthraquinones.

Determination of total phenols : 125 µL of a 1 mg/mL extract is dissolved in 625 µL of Folin-Ciocalteu reagent. After a reaction time of 5 minutes, 500 µL of sodium carbonate (Na_2CO_3) at 75 mg/mL is added. The mixture is vortexed and then incubated in the dark for 2 hours. Absorbance is measured at 760 nm using a T80+ UV/VIS spectrophotometer (Instruments Ltd). The polyphenol content is expressed in mg of gallic acid equivalent per gram of dry extract (Agbodjogbé *et al*., 2022; Koudjina *et al*., (2023); Houngbeme *et al*., 2026).

Determination of flavonoids : To 500 µL of a 2% aqueous aluminium chloride solution, 500 µL of the hydroethanolic extract is added. 3 mL of methanol is then added, followed by incubation for 10 minutes. The optical densities are measured using a spectrophotometer at 415 nm against a reagent blank. Quercetin was used as a reference at a concentration of 10 mg/mL in methanol (Houngbeme *et al*., 2026). The flavonoid concentration by weight is given in mg of quercetin equivalent per gram of dry extract (Maiga *et al*., 2020; Deguenon *et al*., 2023; Houngbeme *et al*., 2026).

Determination of condensed tannins: 500 µL of the extract is added to 1.5 mL of the 4% vanillin solution in methanol, 1.5 mL of concentrated hydrochloric acid and 2 mL of methanol. The catechin blank solution is prepared at 5 mg/mL in methanol (Heimler *et al*., 2006). The mixture is incubated for 15 minutes and the absorbance is measured at 500 nm. The calibration curve is plotted using catechin (0–500 µg/mL). The tannin content is calculated and expressed in µg of catechin equivalent (CAT) per milligram of dry extract (Maiga *et al*., 2020; Houngbeme *et al*., 2026).

Quantification of alkaloids: 1mg of the crude extract was dissolved in dimethyl sulphoxide (DMSO), 1ml of 2N HCl was added and allow to filtered. 5 ml each of bromocresol green and phosphate buffer were addedto the filtrate and transferred to separating funnel. 4 ml of chloroform was added to solution mixtureby vigorous shaking and collected in 10 ml volumetric flask. Then, the volume of these flasks was diluted to themark with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80 and 100 µg/ml) wereprepared in the similar procedure as described above. Absorbance for test and standard solutions were determinedagainst the blank at 470 nm.The total alkaloid content was expressed as mg of AE/g of extract (Vercauteren, 2011).

Determination of saponins: 10 mg of extract is dissolved in methanol to prepare a 10 mg/mL solution. The solution is centrifuged at 10,000 revolutions for 10 minutes. 100 mL of the supernatant is added to 100 mL of a 50% solution of p-anisaldehyde in methanol (volume/volume). A volume of 2 ml of a 50% aqueous sulphuric acid solution (volume/volume) is added to the test tube. The capped test tube is incubated in a water bath at 60 °C for 20 minutes and then placed in an ice bath for 10 minutes. The absorbance of the saponins is measured at 600 nm against a blank prepared under the same conditions. The value obtained, plotted on the protodioscin calibration curve, allows the total saponin content to be estimated.

Determination of terpenes: This involves placing 5 ml of 1% sulphuric acid anisaldehyde in a haemolysis tube; adding 5 ml of the hydro-ethanolic extract; mixing on a vortex mixer; cooling in an ice bath; heating in a water bath at 60° for 20 minutes; then measure the absorbance at 608 nm. The calibration curve is established using different concentrations of linalool prepared from a stock solution. The results obtained are expressed in µg linalool equivalent per ml (µgEL/ml) using the linear regression equation of the calibration curve.

Assessment of antioxidant activity: This was carried out using the direct DPPH (2,2-diphenyl-1-picrylhydrazyl) radical reduction method (Gandonou *et al*., 2018; Agbodjogbé *et al*., 2022; Tokoudagba *et al*., 2026). In solution, DPPH exhibits a

violet colour at 517 nm, which turns yellow when reduced by a free radical scavenger (antioxidant). A stock solution was prepared at 1 mg/mL for the extract and 0.4 mg/mL for the DPPH radical, using analytical-grade methanol. 1.5 mL of the extract solution is then mixed with 3 mL of the DPPH° solution. After 15 minutes in the dark, the optical density is measured at 517 nm. A calibration curve is plotted over the range 0–10 mg/mL using (L)-ascorbic acid supplied by Sigma Aldrich. The percentage reduction in DPPH is calculated using the following equation:

(% DPPH) = 100 × (Abs blank – Abs sample / Abs blank)

Blank Abs: absorbance of the control (reaction mixture excluding the test compounds)

Sample Abs: absorbance of the test compounds.

The IC50 values, which correspond to the concentration of extract that caused a 50% reduction in DPPH radicals, are calculated from the variation in the percentage reduction as a function of the extract concentration.

***In vitro* toxicity test:** The test was carried out in accordance with the standard method used by Fatondji *et al*. (2011); Sakirigui *et al*. (2012a); Sakirigui *et al*. (2012b); and Ombouma *et al*. (2021). Artemia salina L. eggs are incubated in seawater until hatching, for 48 hours. A series of extract dilutions, at progressive concentrations from the stock solution of 100 mg/mL, is placed in contact with 16 larvae each and then left under back-and-forth agitation for 24 hours. Counting the surviving larvae under an electron microscope enables the toxicity of the solution to be assessed. The data obtained were logarithmically transformed and the LC50 was determined. To compare cytotoxic activity based on LC50 values, we used the criteria established in 2014 by Houngbèmè *et al*., according to which an extract is considered non-toxic when the LC50 is ≥ 100 µg/mL.

RESULTS AND DISCUSSION

Qualitative chemical composition: The chemical groups identified in the hydroethanolic extract of the plant are listed in the table below:

Table 1. Chemical groups in the extract

Phytochemical compounds	*Ageratum conyzoides* extract
Tg	+
Tc	++
Ant	++
Fl	++
Leu	+
Al	++
Cr	+
Cm	+
An	+
O-H	-
C-H	+
Tp	++
St	-
Cy	+
Qu	+
Hc	-
Mu	++
Sp	++ (3/10ème)

- (absent); + (present); ++ (abundant)

Al: alkaloids, TC: catechin tannins, TG: gallic tannins, Fl: flavonoids, St: steroids, Tp: terpenes, Leu: leucoanthocyanins, Ant: anthocyanins, Mu: mucilage, O-H: O-heterosides, C-H: C-heterosides; Hl: free glycosides, Cr: reducing compounds; Cm: coumarins, Hc: cardiac heterosides; Qn: quinones, Sp: saponins, Cy: cyanogenic derivatives; An: free anthracenic compounds. From this table, it can be seen that the hydroethanolic extract of Ageratum conyzoides contains 15 of the 18 chemical groups investigated, representing 83.33 per cent of the compounds studied. This demonstrates that the extract of this species is very rich in phytochemicals, which may well account for its wide range of recognised biological activities. The seven (7) most abundant chemical groups are: catechin tannins, anthocyanins, flavonoids, alkaloids, triterpenes, mucilages and saponosides, which possess various pharmacological properties such as wound-healing, anti-inflammatory, antibacterial, antitumour and antiseptic effects (Houngbèmè *et al*., 2014; Tokoudagba *et al*., 2023; Houngbèmè *et al*., 2025). However, the presence of cyanogenic derivatives is a contraindication for the internal use of the plant, due to the recognised toxicity of this chemical group (Mohammedi *et al*., 2011). The presence of triterpenes is consistent with previous studies on Ageratum conyzoides, in which researchers isolated terpenoid molecules. Okwori *et al*. (2006) demonstrated that triterpenes are priority candidate molecules for targeting the strains responsible for chronic wound development, primarily *Pseudomonas aeruginosa*.

Our results therefore align well with those previously reported on the chemical composition of this plant. Indeed, several plant species possess significant antimicrobial activity linked to major chemical groups, including flavonoids, tannins, anthocyanins and, in particular, alkaloids (Lègba *et al*., 2017; Kakpo *et al*., 2019). Steroids are also powerful antioxidants and antimicrobials (Kouchadé *et al*., 2017). Phytochemical screening studies carried out on different types of extracts revealed the presence of hydrolysable and condensed tannins, triterpene saponins and coumarins (Okwori *et al*., 2006; Okunade, 2002), which is consistent with our findings regarding the composition of the hydroethanolic extract. The coexistence of these chemical families confers A. conyzoides with multifaceted biological activity. Chronic wounds are an inevitable source of microbes and inflammatory reactions. Quercetin, a well-known flavonoid, has been identified in the plant's hydro-alcoholic extracts and exhibits remarkable enzyme-inhibiting properties, thereby reducing tissue destruction during the inflammatory phase of wound healing. This action on the proliferation phase, combined with its anti-inflammatory effect during the initial phase, gives A. conyzoides a profile well-suited to chronic or secondarily infected wounds (Pandith *et al*., 2026; Shekhar and Anju, 2012).

Quantitative chemical composition: The quantitative determination of several major metabolites identified in the hydroethanolic extract of Ageratum conyzoides yielded values in the form of equivalent weight concentrations, summarised in Table 2. The values obtained vary considerably. It can be seen that the extract contains the highest saponoside content, at 957.2 ± 2.42 mg protodioscin equivalent/g, followed by condensed tannins, with a content of 491.02 ± 1.03 mg catechin equivalent/g, and alkaloids, with a content of 411.15 ± 10.66 mg atropine equivalent/g. Phytochemicals such as polyphenols, flavonoids, saponins, tannins, alkaloids and terpenes are more abundant in the hydro-ethanolic extract of

Ageratum conyzoides; this may be due to the ethanol-water mixture, as polyphenols generally have a very high affinity for polar solvents and are typically more soluble in hydro-alcoholic mixtures (Houngbeme *et al*., 2026). Hydrolysable tannins are present in greater quantities than condensed tannins in the extract; this is due to the solubility of tannins in water, which varies according to their molecular weight and degree of polymerisation. These results are consistent with those of several authors who have shown that mixed solvents are highly effective at extracting compounds; the use of mixed solvents leads to a high enrichment of the extracts in these compounds. Flavonoids, alkaloids and phenols possess antibacterial, anti-inflammatory and vasodilatory activities. Flavonoids, in particular, show potential for scavenging free radicals and act as antioxidants in biological systems, protecting against inflammation

Table 2. Content of phytochemical compounds per gram of extract

Chemical groups	Content (equivalent)
Saponins	957.2 ± 2,42mgEqP/g
Condensed tannins	491.02 ± 1.03mgEqCAT/g
Flavonoids	380.57 ± 2.15mgEqQ/g
Triterpenoids	310.50 ± 0,26mgEqL/g
Alkaloids	411.15 ± 10,66mgEqA/g
Polyphenols	389.70 ± 0,36 mgEqAG/g

Antioxidant capacity: The percentages of DDPH radical reduction calculated for the reference and for the crude hydroethanolic extract. The linear regression line is plotted based on the variation in the percentage reduction as a function of the concentration of the test compound (Fig. 2). The lower the IC50 value, the greater the antioxidant activity of the extract under test.

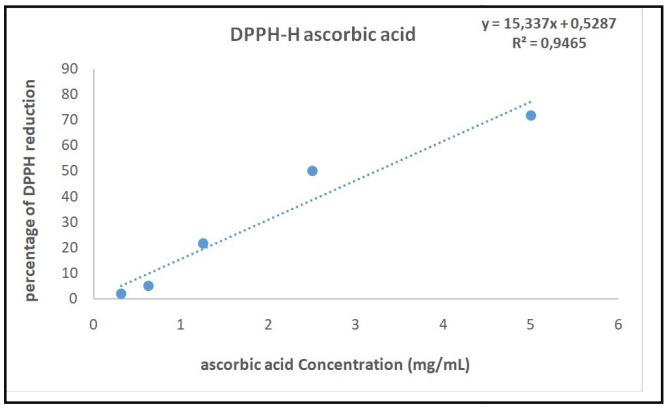

Fig 2. Curve showing the reduction of the DPPH radical by ascorbic acid

It can be seen that the percentage reduction increases with the concentration of the reference standard used. After calculating the IC_{50} value, IC_{50} was found to be 3.22 mg/mL for ascorbic acid and 6.98 mg/mL for the hydroethanolic extract. These results demonstrate that the extract under evaluation possesses very strong antioxidant activity, which does not differ significantly from that of the reference standard used. Several studies have quantified the total phenolic content (TPC) and total flavonoid content (TFC) and established their correlation with antioxidant activity. Islam *et al*. (2013) measured, in the methanolic extract of stems, a total phenolic content of 38.125 ± 2.01 mg/g gallic acid equivalent (GAE) and a total antioxidant capacity of 333.37 ± 4.22 mg/g ascorbic acid equivalent (EAA), with a DPPH IC_{50} of 46.01 ± 2.23 µg/mL (Islam *et al*., 2013). Ozioko *et al*. (2022) demonstrated that the methanolic leaf extract had a high content of flavonoids and phenolic compounds, and that the ethyl acetate fraction, the most polar of the fractions tested, exhibited the best relative antioxidant activity with an IC50 of 0.75 µg/mL. This illustrates the importance of the choice of extraction solvent in optimising biological activities. Antiradical activity is inherent in the major chemical groups, including tannins and flavonoids (Houngbeme *et al*., 2025), as demonstrated by phytochemical screening.

Cytotoxic activity: Figure 3 shows the curve illustrating the variation in the number of dead larvae as a function of the extract concentration. To assess the toxicity of the extract, we calculated the LC50, which is 1.62 mg/mL. This value is well above the set limit of 0.1 mg/mL. Therefore, the extract tested is not toxic to the model cells – namely human carcinoma and lung cells – taking the correlation into account.

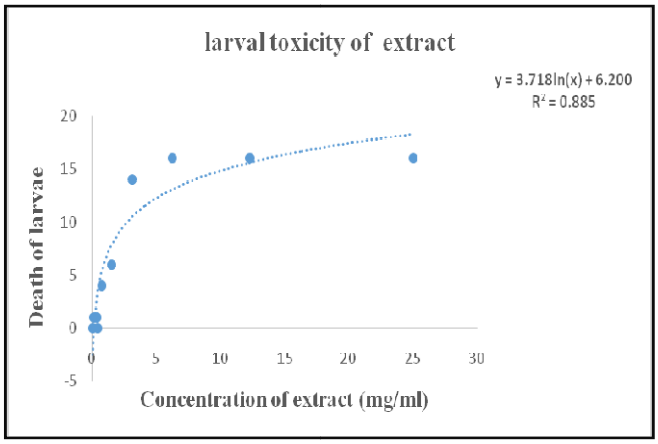

Fig 3. Sensitivity of *Artemia salina* larvae to the extract

Adebayo *et al*. (2010) tested the toxicity of terpenes in rats, and the results indicate an acceptable therapeutic window for topical applications at limited doses. These findings reinforce those for the hydroethanolic extract, which showed no cellular toxicity

CONCLUSION

The hydroethanolic extract of the leafy stems of Ageratum conyzoides L. is a source of antioxidants owing to the diversity and abundance of its secondary metabolites. Its non-toxic nature reinforces the case for developing topical herbal medicines. Further studies may enable this extract to be standardised by precisely identifying new bioactive molecules in the Guinean species.

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