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# Phytochemical Characterization, Antioxidant and Antimicrobial Properties of Hydroethanolic Extract from *Acalypha indica* L. (Euphorbiaceae) Collected in Togo

**Kpakou Mathieu N'Paki [a], Komi Lucien Domedjui [a], Mamatchi Melila [b], Madjouma Kanda [a], Koffi Apeti Gbogbo [a++*], Fousséni Folega [a], Marra Dourma [a], Komlan Batawila [a] and Koffi Akpagana [a]**

*[a] Laboratoire de Botanique et Ecologie Végétale (LBEV) Université de Lomé, Togo.*
*[b] Laboratoire de Génie des Procédés et des Ressources Naturelles (LAGEPREN), Université de Lomé, Togo.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. Authors KAG and MM contributed to design, supervision and manuscript editing, Authors KMN and KLD contributed to experimental studies, data acquisition, data analysis, statistical analysis and manuscript preparation; Authors MK, FF, MD and KB contributed to manuscript review, Author KA conceived the topic and contributed to revising the manuscript. All authors read and approved the final manuscript.*

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*[++] Associate Professor;*
**Corresponding author: E-mail: kagbogbo@gmail.com;*

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## ABSTRACT

Native tropical floras are invaded by non-native species, creating some ecological disorders. Many of them are new reports for local flora but also, they are known to have some medicinal uses in their original regions for human therapy. That seems to be the case of *Acalypha indica* L. (*Euphorbiaceae*). This study aims to provide a synoptic overview of this species in Togolese country and to verify whether the bibliographically reported uses are scientifically verifiable. For this, the species' leafy stem and root were harvested in the Agoè-Nyive 1 district (Lomé), then washed and dried under shade. The samples were crushed and macerated in hydroethanol solvent. Phytochemical screenings were performed. The antioxidant activities were estimated by FRAP method. *In vitro* antimicrobial activity was evaluated using microdilution method. *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Enterococcus faecalis* "resistant strain", *E. coli* "NDM resistant strain", *K. pneumoniae* "Oxa 48 resistant strain", *Trichophyton tonsurans* and *T. mentagrophytes* were tested. Phytochemical screening revealed the presence of phytochemical groups as polyphenols, flavonoids and tannins. The leafy stem showed better antioxidant activity than the root. Hydroethanol extracts showed that leafy stem and root of *A. indica* are microbiostatic against the strains tested.

*Keywords: A. indica; phytoconstituents; antioxidant and antimicrobial activities; resistant strain; microbiostatic.*

## 1. INTRODUCTION

*A. indica* is a plant species recently introduced into Togolese territory; however, according to the documentation it is a potentially important resource for the treatment of many human diseases, particularly infectious ones. This study aims to report its presence on the territory, in order to integrate it into future plant biodiversity management programs. It also assesses the prospects for medicinal uses reported for instance within infectious diseases. Plants have been used for centuries as remedies for human diseases because they contain constituents with therapeutic value [1]. The use of medicinal plants is experiencing a resurgence of interest in both developed and developing countries, attracting the attention of researchers. This situation is leading researchers to take a greater interest in the study of plants [2]. The search for new substances and/or organic compounds is becoming a necessity to alleviate certain problems related to human or animal health [3,4]. In Africa, for example, and particularly in Togo, the use of phytotherapy is a recurrent practice due to the intrinsic value of medicinal plants and the low cost of treatment. Indeed, the Togolese flora has a high biodiversity that benefits endogenous traditional medicine [5]. Among the plant species used in global traditional medicine, *A. indica* can be recognized as one of them. According to Gupta & Tandon [6] and Radji et al. [7], several species of *Acalypha* have medicinal uses. A bibliographical review on the genus shows that its leaves, stems and roots are used in traditional medicine. These parts are mainly used to treat infectious diseases. These species contain chemical compounds as polyphenols, tannins and flavonoids, among others, which prove their antimicrobial activities [8]. In order to confirm the ethnobotanical reports on *A. indica*, and expand the Togolese flora used for health care, it was considered necessary to carry out a phytochemical study using root and leafy stem hydro-ethanolic extracts to evaluate *In vitro* antioxidant and antimicrobial activities based on six bacterial strains and two fungal strains, known as human pathogens.

## 2. MATERIALS AND METHODS

### 2.1 Solvents, Reagents and Standards

Dragendorff's reagent, concentrated HCl, Fehling's lye, ferric chloride reagent, NaOH, concentrated sulphuric acid, Molisch's reagent, Folin-Ciocalteu reagent, sodium carbonate, $NaNO_2$, aluminium trichloride hexahydrate ($AlCl_3$, $6H_2O$), gallic acid, quercetin, catechin, ammoniacal ferrous sulphate ($NH_4Fe(SO_4)_2$) BuOH/HCl, Ferric Reducing Antioxidant Power (FRAP) solution, ferrous sulphate solution ($FeSO_4$, $7H_2O$), methanol, 95 % ethanol, chloramphenicol, gentamicin and griseofulvin, Sabouraud chloramphenicol agar, PDA agar, Mueller Hinton agar and peptone broth were used in this study.

## 2.2 Microbial Strains

Organisms used in this study were *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Klebsiela pneumoniae* ATCC 700603, *Enteroccocus faecalis* "resistant strain", *Escherichia coli* "NDM resistant strain", *Klebsiela pneumoniae* "Oxa 48 resistant strain", *Trichophyton tonsurans* and *Trichophyton mentagrophytes.*

## 2.3 Collection and Identification of Plant Materials

The method used during the floristic inventory was random sampling through an inventory carried out in the five ecological zones of the country with particular emphasis on the ecological zone 5 of the Coastal Plains. Ecological and floristic monitoring was based on the last two years (2021 and 2022). The collected samples identification was carried out using [9] and [10]. The data were refined by references available on the Kew Botanic Gardens's Plants of the World Online. Vouchers of collected samples were deposited in the herbarium of the Department of Botany (University of Lomé).

The roots were separated from the leafy stems, and then the two parts were washed and dried under shade for two weeks at laboratory temperature. The roots and leafy stems were reduced to powder using an electric mill; the powders were then submitted to extraction.

## 2.4 Preparation of Extracts

One hundred grams of powdered *A. indica* root and leaf stem were macerated in 1000 ml of hydroethanol solution (v/v) with stirring for 72 hours. The macerates were then filtered through sieves and Whatman No. 2 paper and concentrated in a BUCHI Rotavapor at 45°C. Finally, the extracts were transferred to sterile bottles and stored at 4°C until further analysis.

## 2.5 Qualitative Phytochemical Analysis

The major phytochemical groups such as alkaloids, flavonoids, tannins, polyphenols, saponins, triterpenes and sterols, total carbohydrates, coumarins, reducing sugars, cardiac glycosides and free quinones were examined in the hydroethanol extracts of the root and leafy stem of *A. indica*. The presence of these chemical phytoconstituents was evidenced either by precipitation reactions and/or staining of the reaction medium.

## 2.6 Detection of Alkaloids

A few drops of Dragendorff reagent were added to 1 mL of each extract. The appearance of an orange-red precipitate confirms the presence of alkaloids in the extracts analyzed [11].

## 2.7 Detection of Flavonoids

2 mL methanol was added to 2 mL of each extract, followed by a few drops of sodium hydroxide solution (1/10). The appearance of yellow-orange coloration characterized the presence of flavonoids in the extracts analyzed [11].

## 2.8 Identification of Tannins

2 mL distilled water followed by three (03) drops of 1% iron perchloride ($FeCl_3$) were added to 2 mL of each extract. After two (02) minutes incubation, the presence of gallic tannins was revealed by blue, dark-blue or black coloration. In addition, green to dark green coloration indicated the presence of catechic tannins in the extracts analyzed [12].

## 2.9 Detection of Polyphenols

2 mL methanol and a few drops of 1% iron perchloride ($FeCl_3$) were added to 2 mL of each extract. The presence of a blue-black color indicates the presence of phenolic compounds in the extracts analyzed [13].

## 2.10 Detection of Saponins

2 mL of each extract was taken and shaken vigorously for 1 minute. The presence of persistent foam for 15 minutes indicates the presence of saponins in the extracts analyzed [12].

## 2.11 Detection of Triterpenes and Sterols

1.6 mL of chloroform followed by three (03) drops of concentrated sulfuric acid were added to 4 mL of each extract. The appearance of a reddish-brown ring between the light and dark phases indicates a positive test [12].

## 2.12 Detection of Coumarins

2 mL distilled water and 200 µL ammonia were added to 4 mL of each extract. The appearance of fluorescence under ultraviolet light signals the presence of coumarins in the extracts analyzed [14].

## 2.13 Identification of Reducing Compounds

500 µL of Molish reagent was added to 1 mL of each extract. The mixture was then thoroughly homogenized and poured into 2 mL of concentrated sulfuric acid solution. The formation of a ring at the interface of the two (02) solutions revealed the presence of reducing sugars in the extracts analyzed.

## 2.14 Identification of Total Carbohydrates

To 1mL of extract were added 500 µL of Molisch reagent (α-naphthol dissolved in ethanol). After mixing, 1mL of concentrated sulfuric acid is slowly added to the sides of the inclined test tube, without mixing, to form a layer. A positive reaction is indicated by the appearance of a purple ring at the interface between the acid and the sugars [15].

## 2.15 Detection of Cardiotonic Glycosides

2 mL of Chloroform were added to 1 mL of the aqueous powder solution, the appearance of a reddish-brown coloration after the addition of sulfuric acid ($H_2SO_4$) indicates the presence of cardiac glycosides [16].

## 2.16 Detection of Free Quinones

The appearance of purplish-red coloration following the addition of a few drops of sodium hydroxide solution (1/10) to 2 ml of extract, reflected the presence of free quinones in the extracts analyzed [14].

## 2.17 Quantitative Phytochemical Analysis

The contents of total phenolics, total flavonoids and total tannins in the hydroethanolic extracts of the leafy stem and root were determined by UV-visible spectrophotometry.

## 2.18 Total Polyphenols Content

Total polyphenols were determined according to Singleton et al. [17] method. 200 µl of the sample to be determined (gallic acid or 1 mg/ml extract) and 500 µl of Folin-Ciocalteu reagent (diluted ½ in distilled water) were added to each test tube. After 5 minutes of reaction, 500 µL of sodium carbonate (20 g/l) was added. The volume of the previous mixture was made up to 4 ml with distilled water. After shaking, the different solutions were incubated for 30 minutes at laboratory temperature in the dark. The optical density was read at 760 nm using a METASH UV-visible spectrophotometer (UV-5200 PC) equipped with MetaSpec Pro data acquisition software, against a blank obtained by mixing 500 µl of Ciocalteu Folin Reagent (CFR), 500 µl of sodium carbonate and 200 µl of distilled water. The equation of the calibration curve obtained from successive dilutions of the gallic acid stock solution (200 mg/l) gives the result corresponding to the content of total phenolic compounds, expressed as mg Gallic Acid Equivalent (GAE)/g dry extract.

## 2.19 Total Flavonoids Content

Flavonoids were determined by the aluminium trichloride method described by Zhishen et al. [18] with some modifications. In a glass haemolysis tube, 800 µl of extract or standard, or distilled water for control, was added to 240 µl of 5 % $NaNO_2$. After incubation for 6 minutes at room temperature, 240 µl of 10 % hydrated hexaaluminium trichloride ($AlCl_3$, $6H_2O$) was added. After six minutes, 1.6 L of 1 M NaOH was added. Absorbance was read immediately at 510 nm against the control. A calibration curve was established with quercetin at different concentrations between 0 and 1000 µg/ml under the same conditions as the samples. The results obtained were expressed as mg quercetin equivalent (QE)/g dry extract.

## 2.20 Total Tannins Content

Proanthocyanidins (condensed tannins) were determined by the butanol/hydrochloric acid (BuOH/HCl) method [19]. Briefly, 0.2 ml of an ammoniacal iron sulphate solution ($NH_4Fe(SO_4)_2$: 20 g/l) was added to a test tube, followed by 7 ml of BuOH/HCl (95 % - 5 % ; v/v) and 0.2 mL of extract (30 mg/ml). The mixture was incubated in a water bath at 95°C for 40 minutes. A pink or red colour was obtained. The proanthocyanidin concentrations of the samples were determined by reading the optical density (OD) at a wavelength of 550 nm using a METASH UV-visible spectrophotometer (UV-5200 PC). In

order to derive the proanthocyanidin content (Tp) of the samples, expressed as mg catechin equivalent (CE)/g dry extract, the following formula was used for the calculations according to Aksamit-Stachurska et al. [20]. : Tp=DO/0.280 with OD = 0.280 corresponding to 1% catechin. Catechin was therefore used as the standard molecule.

## 2.21 Determination of Antioxidant Activity

The protocol of Nair et al [21]. was used in this study. To test tubes containing 3 mL of freshly prepared FRAP solution (25 ml acetate buffer + 2.5 ml 10 mM Fe3+-TPTZ [2,4,6-Tris (2-pyridyl)-s-triazine] prepared in a 40 mM HCl solution + 2.5 mL 20 mM $FeCl_3$-$6H_2O$), 10 µl of sample was added. The reading was taken at 593 nm on a METASH UV-visible spectrophotometer (UV-5200 PC) against the blank after 10 min incubation. A calibration curve was constructed from a concentration range (0-1800 µM) of a solution of iron sulphate ($FeSO_4$, $7H_2O$) dissolved in methanol. The result was expressed as µmol $FeSO_4$ equivalent/mg dry extract.

## 2.22 Antimicrobial Activity

### 2.22.1 Preparation of hydroethanol extracts and antibiotics

700 mg of hydroethanol extracts from the root and aerial parts were dissolved in 7 ml of hydroethanol solution (v/v) and filtered once through a sterile 0.20 µm Millipore filter under aseptic conditions. The extracts were then placed in sterile Falcon tubes and stored at 4°C for future use. Chloramphenicol 1 mg/ml (1%) and griseofulvin 20 µmg/ml were prepared as reference antibiotics for the bacteria and fungi tested.

### 2.22.2 Preparation of inocula

Bacterial inocula were prepared from Müller-Hinton broth and incubated for 24 hours. Bacterial suspensions with a turbidity of 0.5 Mac Farland ($4.10^8$ CFU/ml) were prepared using sterile physiological water (NaCl; 0.9 %). Conidial suspensions of *Trichophyton tonsurans* and *Trichophyton mentagrophytes* were prepared from 7-day-old cultures on PDA agar incubated at 25°C. Conidia were harvested with sterile physiological water (NaCl; 0.9%). Appropriate dilutions were made from these conidial suspensions to prepare 0.5 Mac Farland turbidity fungal inocula.

### 2.22.3 Antimicrobial activity

Minimum inhibitory concentrations (MICs), minimum bactericidal concentrations (MBCs) and minimum fungicidal concentrations (MFCs) were determined using the microdilution method described by Noumedem et al. [22] and Anani et al. [23] with some modifications. For each experiment, a sterility control (peptone broth and extract), a negative control (inoculum and antibiotic) and a positive control (inoculum and peptone broth) were included. A Microwell 96-well plate was used for the test and 100 µl of peptone broth was added to each well. Next, 100 µl of 200 mg/mL extract was added to the peptone broth in each well, followed by 100 µl of inoculum. This second order regressive dilution gave final concentrations of 50, 25, 12.5, 6.25 and 3.125 µg/ml. Bacteria were incubated at 37°C for 24 h and at 25°C for 7 days for dermatophytes. The MIC was defined as the lowest extract concentration at which the tested fungal strain showed no visible growth. Minimum microbiocidal concentrations (MMC) were determined by plating 10 µl aliquots from wells showing no microbial growth on Mueller-Hinton or PDA agar and incubating at 37°C for 24 hours for bacteria and at 25°C for 7 days for dermatophytes. The antibiotic potency (AP) is considered microbiocidal if MFC/MIC ≤ 1, microbiostatic if MFC/MIC > 1. The test has been repeated twice under aseptic conditions.

## 2.23 Statistical Analysis

All data were integrated in an Excel™ 2016 spreadsheet, and processed with Graphpad Prism™ 8.4.3 for statistical analysis.

# 3. RESULTS

## 3.1 Status of the Species in Togo

For several decades, there has been an upsurge in Togo of some recently introduced species in Togo such as *Croton hirtus* L'Hérit [24]. One of these famous species newly introduced in the Togolese flora, was *Chromolaena odorata* (L.) R. M. King & Robinson [25]. This is also the case of *A. indica*. Several reasons as climate change, increasingly poor soils, ineffective border control by plant protection services, etc., could be point out. Weeds have a very high reproductive capacity, which allows them quickly colonize any suitable ecosystem. *A. indica* is an annual (but can be multi-annual) sub-woody plant. In Togo, it is widespread on the coastal strip up to approximately 20 kilometers inland (Fig 1). The

climate is subequatorial Guinean type with two rainy seasons, and precipitation amounts varying between 700 and 1000 mm per year (Fig 2). Relative humidity is constantly high (> 50%) throughout the year and temperatures vary from 25° to 35°C [26].

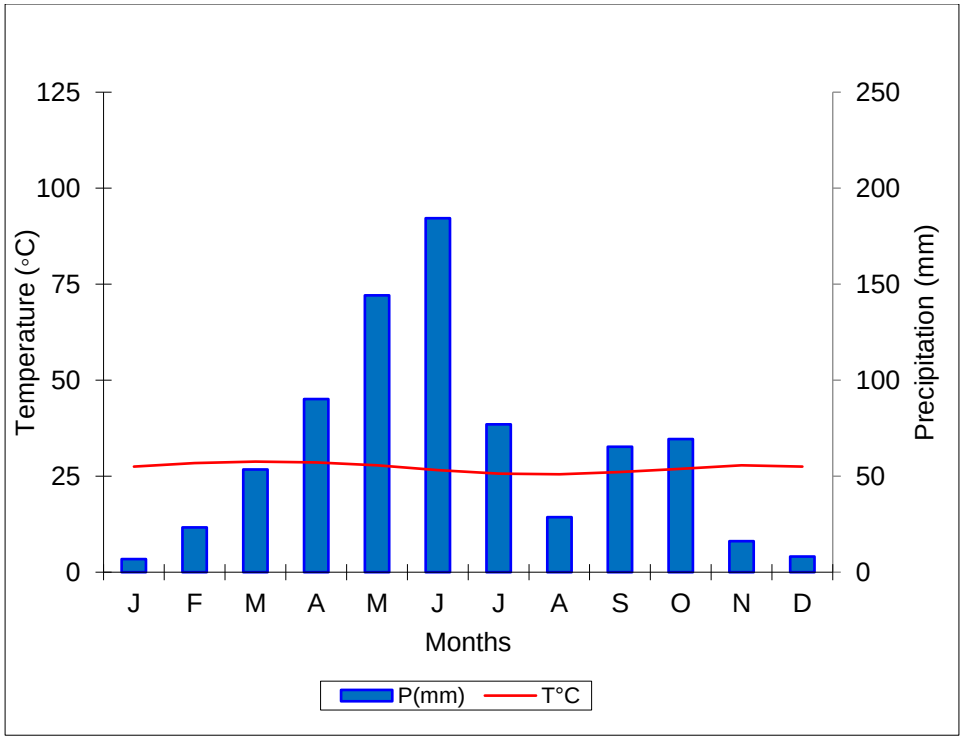


**Fig. 1. Ombrothermal curve of Lomé climatic station (1970-2020)**


**Fig. 2. Some shapes of *A. indica* collected in Togo**

*a. In settlement; b. Some mature individuals; c. Leaves at the top revealing the phyllotaxis; d. An individual near an Amaranthus spinosus L. (Amaranthaceae)*

Soils are of two shapes. They are less evolved, with a variety of texture depending on the location. They are located along the coastline (strip 2 to 5 kilometers wide) where they are developed on sandy sedimentary materials. Their texture and water properties are deficient. Tropical ferruginous sandy-clay to clay soils also exist with good physicochemical capacities.

In the carried-out surveys on fallows, *A. indica* is often accompanied by *Achyranthes aspera* L. (Amaranthaceae), *Boerhavia diffusa* L. (Nyctagynaceae), *Euphorbia hirta* L. (Euphorbiaceae), *Sida acuta* Burm. f. (Tiliaceae), *Portulaca oleracea* L. (Portulacaceae), *Corchorius aestuans* L. (Tiliaceae), *Indigofera tinctoria* L. (Papilionaceae), *Cyperus rotundus* L. (Cyperaceae), *Dactyloctenium aegytium* (L.) Willd. (Graminae) and *Alternanthera pungens* Kunth (Amaranthaceae). It is a ruderal species now common in gardens, fallows, vacant lots, along paths, roads and in house yards. It is gradually becoming a weed for market garden crops in the area.

According to https://idao.cirad.fr/content/adventoi/especes/a/accin/accin_fr.html (accessed in 10 November 2023), *A. indica* is an erect semi-woody plant, reaching 1.5 m in height. It has a taproot and its stem is ribbed, branching near the base. The leaves, light green, are simple, alternate, long-petiolate; they are broadly oval, 1.2 cm to 6.5 cm × 1 cm to 4 cm. The base of the leaves is rounded to briefly attenuate. The leaf margin is crenulated-serrated with an acute or obtuse apex. The petiole is 1.5 to 5.5 cm long. It has catkin-shaped inflorescences with cup-shaped involucres surrounding the tiny flowers. The branched inflorescences develop in the leaf axils. The female flowers, at the base, are in small green leafy cups with a toothed edge. The male flowers are very small and gathered at the end of the inflorescence (Fig 2). The pollen is roughly rounded and measures 10 to 12 µ in diameter. The fruit is a capsule, often decorated with excrescences, with three cells, each containing a seed.

In Togo, samples of *A. indica* were collected in November 2022 in the Southern part of the country especially in the coastal districts of Golfe, Agoè-Nyivé and Lacs (Fig 3). The identification of the species was confirmed (using particularly Google Lens™) at the herbarium of the Laboratory of Botany and Plant Ecology (University of Lomé) where samples were deposited.

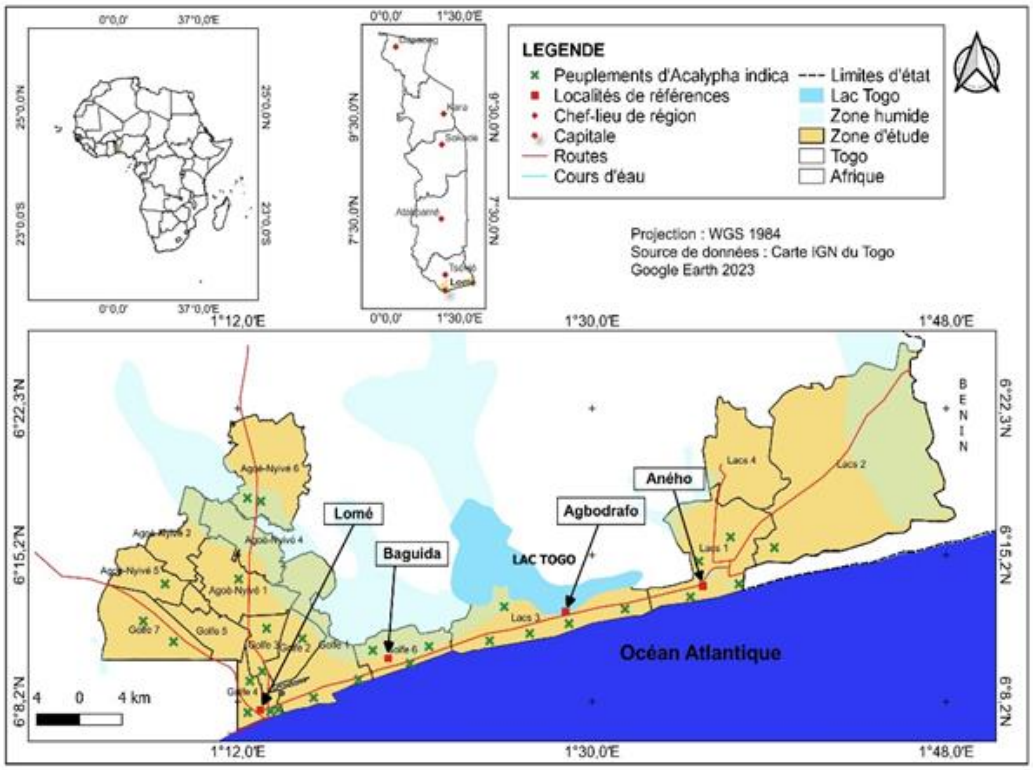


**Fig. 3. Sites on witch *A. indica* populations were observed and confirmed in Togo**

### 3.2 Phytochemical Constituents

Table 1 presents the results of the qualitative phytochemical analyses of the hydroethanolic extracts of the leafy stem and root of *A. indica*. The leafy stem and root contain the same phytochemical groups such as alkaloids, reducing compounds, polyphenols, tannins, cardiotonic heterosides, flavonoids, coumarins, triterpenes and sterols, saponins, total carbohydrates, free quinones.

### 3.3 Total Polyphenols, Flavonoids and Tannins Content

The contents of total polyphenols, total flavonoids and total tannins (catechin and gallic) in the hydroethanolic extracts of the leafy stem and root of *A. indica* are shown in Table 2. Analysis of the data showed that the leafy stem contained 94.8 mg GA of total polyphenols, 271.01 mg QE of total flavonoids and 37.15 mg CE of total tannins per g of sample dry hydroethanolic extract. In addition, the hydroethanol extract of the root contained 61.39 mg GAE of total polyphenols, 149.15 mg QE of total flavonoids and 23.62 mg CE of total tannins. In a comparative approach, the contents of total polyphenols, total flavonoids and total tannins in the leafy stem of *A. indica* L. were each higher than those measured in the root.

### 3.4 Antioxidant Activities

The results of the antiradical activities and EC50 of the hydroethanol extracts of the leafy stem and root of *A. indica* are shown in Table 3. The analysis shows that the leafy stem had better (236.98 ± 3.00 µg/ml) antiradical activity compared to that of the root (216.08 ± 1.86 µg/ml) and quercetin (158.93 ± 3.13 µg/ml).

**Table 1. Phytochemical constituents of hydroethanolic extracts**

| Phytochemical Groups | Leafy Stem | Root |
|---|---|---|
| Alkaloids | + | + |
| Reducing Compounds | + | + |
| Polyphenols | + | + |
| Tannins | + | + |
| Cardiotonic Heterosides | + | + |
| Flavonoids | + | + |
| Coumarins | + | + |
| Triterpenes and Sterols | + | + |
| Saponins | - | - |
| Total Carbohydrates | + | + |
| Free Quinones | + | + |

*Indications : + = present ; – = not detected*

**Table 2. Total polyphenols, total flavonoids and total tannins in hydroethanol extracts of *A. indica* L. leafy stem and root**

| Molecules Sought | Concentration/g Dried Leafy Stem Hydroethanol Extract | Concentration/g Dried Root Hydroethanol Extract |
|---|---|---|
| Total polyphenols | 94.8 mg GAE | 61.39 mg GAE |
| Total flavonoids | 271.01 mg QE | 149.15 mg QE |
| Total tannins | 37.15 mg CE | 23.62 mg CE |

*NB: GAE = Gallic Acid Equivalent; QE = Quercetin Equivalent; CE = Catechin Equivalent*

**Table 3. Free radical scavenging activity of hydroethanol extracts of leafy stem and root of *A. indica* L. using the FRAP test**

| Parameters | Quercetin | Leafy Stem | Root |
|---|---|---|---|
| EC 50 (µg/ml) | 158.93 ± 3,13 | 236.98 ± 3,00 | 216.08 ± 1,86 |

**Table 4. Antimicrobial activity of hydroethanol extracts of *A. indica L.* leafy stem and root**

| Microbial strains | MIC (mg/ml) | | MBC or MFC (mg/ml) | | AP | |
|---|---|---|---|---|---|---|
| | Ro | Ls | Ro | Ls | Ro | Ls |
| *S. aureus* ATCC 25923 | 25 | 25 | > 25 | >25 | >1 | >1 |
| *E. coli* ATCC 25922 | 25 | 25 | >25 | >25 | >1 | >1 |
| *E. coli* (NDM Rs) | 25 | 25 | >25 | >25 | >1 | >1 |
| *K. pneumoniae* ATCC 700603 | 25 | 6.25 | >25 | 25 | >1 | 4 |
| *K. pneumoniae* (Oxa 48 Rs) | 25 | 25 | >25 | >25 | >1 | >1 |
| *E. faecalis* (Rs) | 25 | 6.25 | >25 | 12.5 | >1 | 2 |
| *T. mentagrophytes* | 25 | 25 | >25 | >25 | >1 | >1 |
| *T. tonsurans* | 25 | 25 | >25 | >25 | >1 | >1 |

*Ro: Acalypha Indica Root; Tf : Acalypha Indica Leafy Stem; MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration; MFC: Minimum Fungicidal Concentration; AP: Antibiotic Potency; Rs : Resistant Strain*

### 3.5 Antimicrobial Activity

Antimicrobial tests were carried out to determine the MIC, MBC, MFC and AP of hydroethanolic extracts of the leafy stem and root of *A. indica*. The results are presented in Table 4. Analysis of this table shows that all the microbial strains tested were sensitive to the extracts tested. The root extract had MIC values of 25 mg/mL and BMC or MFC values above 25 mg/ml. For the leafy stem extract, MIC values ranged from 25 to 6.25 mg/mL, while BMC or MFC values ranged from 12.5 to 25 mg/ml. The antibiotic activity of the hydroethanol extracts of the root and leafy stem of *A. indica* against the strains tested was considered to be microbiostatic.

## 4. DISCUSSION

The species is widely distributed in the tropics of the Old World. In West Africa, it has been reported in Nigeria. It is also found in the Indian Ocean islands, India, Southeast Asia, Yemen and Oceania. It was introduced to the New World tropics.

In West Africa, [9] cited fourteen species of *Acalypha* among which four are (*A. ciliata* Forssk., *A. crenata* Hochst. ex A. Rich., *A. racemosa* Wall. ex Baill. et *A. segetalia* Müll. Arg.) are inventoried in Togo [10].

Many uses in traditional medicine have been reported in Madagascar, Mauritius, Seychelles, Reunion and East Africa where the plant species is intervenes in the treatment of skin and respiratory ailments. Root decoction is taken against asthma and stomach aches (https://www.pharmacopoeia.com/). However, as with any other species of the Euphorbiaceae family, it is advisable to be very careful and avoid any hazardous use [27-31]. This herb is held in very high esteem in traditional Tamil Siddha medicine, as it is believed to rejuvenate the skin.

Analysis of the phytochemical constituents of the hydroethanolic extracts of the leaf stem and root of *Acalypha indica* L. revealed the presence of the major phytochemical groups such as alkaloids, reducing compounds, polyphenols, tannins, cardiotonic heterosides, flavonoids, coumarins, triterpenes and sterols, saponins, total carbohydrates, free quinones. This result shows that all the major chemical groups we were looking for were present at all levels of the plant. In their study, Kusrini et al. [32]. also reported that *A. indica* contains secondary metabolites such as alkaloids, tannins, saponins, glycosides, steroids, phenolics, terpenoids and flavonoids. The results of the present study on the qualitative analysis of the extracts of the considered *A. indica* L. organs are also in agreement with those reported by Mohan et al. [33] who, on the other hand, reported the absence of terpenoids, reducing sugars and amino acids in the hydroethanolic extracts of *A. indica* L. leaves. This difference may be related to biophysical factors at the sites where the plant samples were harvested. Furthermore, in their preliminary phytochemical study, Godipurge et al. [34] reported the presence of glycosides, saponins, terpenoids, tannins, flavonoids and phenolic compounds. According to the latter, these chemical groups may be responsible, at least in part, for the analgesic and anti-inflammatory effects of this species. The presence of bioactive molecules such as phenols, alkaloids, steroids and terpenoids in *A. indica* extracts would justify its use in the treatment of a number of ailments. According to

Dineshkumar et al. [35], the parts of *A. indica* used for therapeutic purposes are the leaves, roots, stems and flowers. This plant is used in the treatment of pneumonia, asthma, rheumatism and several other ailments, as well as being an emmenagogue.

The determination of total polyphenols, total flavonoids and total tannins (catechin and gallic) in the hydroethanolic extracts of the leafy stem and root of *A. indica* showed that they were present in different proportions depending on the part of the plant used. Furthermore, the results showed that the hydroethanolic extracts of the leafy stem and root of *A. indica* contained more total flavonoids, followed by total polyphenols and total tannins. The low levels of bioactive molecules in the roots compared to the leafy stem of the plant could be explained by the anatomical structure of the roots, stem and leaves of the plant. Indeed, the aerial organs of plants are rich in chlorophyll parenchyma, which contains epidermal cells (secretory tissues) capable of producing and accumulating secondary metabolites in their cytoplasm. This would explain the high levels of bioactive molecules in the leafy stem. Secretory tissues are not very abundant in plant roots in favour of reserve cells. The contents obtained in this study are higher than those reported by Godipurge et al. [34]. These authors reported that the highest contents of phenolic compounds and total flavonoids were 9.27 mg TA/g and 8.75 mg Ru/g, respectively, in the polyphenolic fraction of the ethanolic extract of *A. indica*. Kusrini et al. [32] also reported that the total phenolic content in the ethanolic extract of *A. indica* L. was 203.07 GAE/g. This variation in the content of biomolecules in the extracts may be related to the biophysical factors of where the plant samples were collected, the extraction solvents and the extraction method. Phenolic compounds and flavonoids are important components of the plant and some of their pharmacological effects could be attributed to the presence of these phytoconstituents [34]. Potential biological properties such as antibacterial, cytotoxic, antimutagenic, antitumour, antidiabetic, antiviral, antibacterial and antifungal properties have been associated with the various compounds of *A. indica* extracts [36].

The antioxidant properties of natural substances can be assessed using various methods, including the FRAP test [37]. Analysis of the results of the present study revealed that the hydroethanol extract of leafy stems showed the best antiradical activity. According to Kpètèhoto et al. [37], total tannins, total flavonoids and total polyphenols contained in the extracts are molecules with antibacterial and antioxidant activities. Furthermore, Kang et al. [38] reported that the polar polyphenolic molecules contained in plant extracts contribute to an increase in their anti-free radical activity. This observation corroborates the results of this study, which showed that the total phenolic content of the root (61.39 mg GAE/g) was lower than that of the leafy stem (94.8 mg GAE/g). This could therefore explain the high antioxidant activity of the hydroethanol extract of *A. indica* leafy stems compared to that of the roots. Previous studies have already investigated the anti-free radical activity of *A. indica* extracts. Indeed, Marwah et al. [39] reported an antiradical activity of 81.6 ± 0.4 % for the hydroethanol extract of the whole plant using the DPPH method, after incubation for 15 minutes at a concentration of 50 µg/ml. In addition, hexane, chloroform and methanol extracts also showed significant antioxidant activity with IC50 of 6.19, 5.70 and 7.79 mg/ml respectively using a DPPH radical reduction assay and with IC50 of 6.13, 6.31 and 6.37 mg/ml respectively using the ABTS radical reduction assay [40]. A good correlation (correlation coefficient, r = 0.812) between antioxidant activity and total phenolic compounds in wound healing plants including *A. indica* was also reported [41].

The microbiostatic properties demonstrated in this study justify the use of *A. indica* in traditional medicine. The antimicrobial activity of plant extracts is related to a synergistic effect between the different chemical groups present in plants, i.e. alkaloids, tannins and flavonoids, all of which have antimicrobial activity [8,42-44]. The antimicrobial activity of *A. indica* leaves against bacterial strains including *S. aureus*, *K. pneumoniae* and *E. coli* were evaluated [41]. The results showed that hexane, chloroform, ethyl acetate and methanol extracts had MIC values of 0.312, 0.625, 0.312 and 0.156 mg/mL against *S. aureus* respectively. In contrast, the MIC values against *K. pneumoniae* and *E. coli* were > 5 mg/ml. The MICs of the hydroethanolic extracts of the leafy stem and root of *A. indica* L. against *Trichophyton mentagrophytes* and *Trichophyton tonsurans* are very high compared to those of Noumedem et al. [22]. who reported that the methanolic extract of *A. manniana* leaves had antifungal activity against *T. mentagrophytes*, *T. equinum* and *T. terrestre* with MIC values of 0.25, 0.25 and 0.51 mg/mL, respectively. This could be

explained by the biomolecular content of each type of extract and the sensitivity of the strains tested.

## 5. CONCLUSION

This study made it possible to take stock of the presence of *A. indica* in Togo. Its main chemical constituents have been identified as well as its antioxidant and antimicrobial properties. The species is present in the southern part of the country. The major chemical groups are represented instead of saponins. The leafy stems were more provided in polyphenols and have good capacity to reduce iron ions and free radicals. The species is therefore a potential source of antioxidant phytoconstituents. This can justify its use in phytomedicine for the treatment of diseases caused by oxidative stress. The antibiotic effect of hydroethanolic extracts of leafy stems and roots was evaluated as microbiostatic; which could justify the use of this species in traditional medicine. Furthermore, the leafy stem seems more suitable for use in traditional phytomedicine.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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