PHYTOCHEMICAL CARACTERISATION, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF THE HYDROETHANOLIC EXTRACT OF *ACALYPHA INDICA* L. (EUPHORBIACEAE) COLLECTED IN TOGO

UNDER PEER REVIEW

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 (Lome), 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.

23 **Key Words**: flora of Togo, new report, *A. indica*, phytoconstituents, antioxidant and
24 antimicrobial activities.

25

UNDER PEER REVIEW

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 (Mohammedi, 2006). 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 (Lipkus et al., 2008). The search for new substances and/or organic compounds is becoming a necessity to alleviate certain problems related to human or animal health (Balenghien et al., 2007; Fialkowski et al., 2005). 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 (Françoise et al., 2018). Among the plant species used in global traditional medicine, *A. indica* can be recognized as one of them. According to Gupta & Tandon (2004) and Radji *et al*. (2010), 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 (Athamena et al., 2010). 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

UNDER PEER REVIEW

49 hydro-ethanolic extracts to evaluate *in vitro* antioxidant and antimicrobial activities based on six
50 (6) bacterial strains and two (2) fungal strains, known as human pathogens.

51 **MATERIALS AND METHODS**

52 **Solvents, reagents and standards**

53 The following were used in this study:

54 Dragendorff's reagent, concentrated HCl, Fehling's lye, ferric chloride reagent, NaOH,
55 concentrated sulphuric acid, Molisch's reagent, Folin-Ciocalteu reagent, sodium carbonate,
56 NaNO2, aluminium trichloride hexahydrate (AlCl3, 6H2O), gallic acid, quercetin, catechin,
57 ammoniacal ferrous sulphate (NH4Fe(SO4)2) BuOH/HCl, FRAP solution, ferrous sulphate
58 solution (FeSO4, 7H2O), methanol, 95 % ethanol, chloramphenicol, gentamicin and
59 griseofulvin, Sabouraud chloramphenicol agar, PDA agar, Mueller Hinton agar and peptone
60 broth.

61 **Microbial strains**

62 A total of eight (8) human pathogenic microorganisms were tested, including six (6) bacteria and
63 two (2) dermatophytes. These were *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC
64 25922, *Klebsiela pneumoniae* ATCC 700603, *Enteroccocus faecalis* "resistant strain",
65 *Escherichia coli* "NDM resistant strain", *Klebsiela pneumoniae* "Oxa 48 resistant strain",
66 *Trichophyton tonsurans* and *Trichophyton mentagrophytes.*

67 **Collection and Identification of plant material**

68 The method used during the floristic inventory was random sampling through an inventory
69 carried out in the five (5) ecological zones of the country with particular emphasis on the

UNDER PEER REVIEW

70 ecological zone 5 of the Coastal Plains. Ecological and floristic monitoring was based on the last
71 two (2) years (2021 and 2022). The collected samples identification was carried out using
72 Hutchinson and Dalziel (1958), and Brunel et al. (1984). The data were refined by references
73 available on the Kew Botanic Gardens's Plants of the World Online. Vouchers of collected
74 samples were deposited in the herbarium of the Department of Botany (University of Lome).

75 The roots were separated from the leafy stems, then the two parts were washed and dried under
76 shade for two (2) weeks at laboratory temperature. The roots and leafy stems were reduced to
77 powder using an electric mill; the powders were then submitted to extraction.

78 **Preparation of extracts**

79 One hundred (100) grams of powdered *A. indica* root and leaf stem were macerated in 1000 ml
80 of hydroethanol solution (v/v) with stirring for 72 hours. The macerates were then filtered
81 through sieves and Whatman No. 2 paper and concentrated in a BUCHI Rotavapor at 45°C.
82 Finally, the extracts were transferred to sterile bottles and stored at 4°C until further analysis.

83 **Qualitative phytochemical analysis**

84 The major phytochemical groups such as alkaloids, flavonoids, tannins, polyphenols, saponins,
85 triterpenes and sterols, total carbohydrates, coumarins, reducing sugars, cardiac glycosides and
86 free quinones (Elzagheid, 2018; Khaldi et al., 2012; Khitri et al., 2016; Mohan et al., 2012) were
87 examined in the hydroethanol extracts of the root and leaf stem of *A. indica*. The presence of
88 these chemical phytoconstituents is evidenced either by precipitation reactions and/or staining of
89 the reaction medium.

90

UNDER PEER REVIEW

91 **Quantitative phytochemical analysis**

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

94 **Total polyphenols content**

95 Total polyphenols were determined according to Singleton *et al*. (1999) method. 200 µl of the
96 sample to be determined (gallic acid or 1 mg/ml extract) and 500 µl of Folin-Ciocalteu reagent
97 (diluted ½ in distilled water) were added to each test tube. After 5 minutes of reaction, 500 µL of
98 sodium carbonate (20 g/l) was added. The volume of the previous mixture was made up to 4 ml
99 with distilled water. After shaking, the different solutions were incubated for 30 minutes at
100 laboratory temperature in the dark. The optical density was read at 760 nm using a METASH
101 UV-visible spectrophotometer (UV-5200 PC) equipped with MetaSpec Pro data acquisition
102 software, against a blank obtained by mixing 500 µl of Ciocalteu Folin Reagent (CFR), 500 µl of
103 sodium carbonate and 200 µl of distilled water. The equation of the calibration curve obtained
104 from successive dilutions of the gallic acid stock solution (200 mg/l) gives the result
105 corresponding to the content of total phenolic compounds, expressed as mg Gallic Acid
106 Equivalent (GAE)/g dry extract.

107 **Total flavonoids content**

108 Flavonoids were determined by the aluminium trichloride method described by Zhishen *et al.*
109 (1999) with some modifications. In a glass haemolysis tube, 800 µl of extract or standard, or
110 distilled water for control, was added to 240 µl of 5 % $NaNO_2$. After incubation for 6 minutes at
111 room temperature, 240 µl of 10 % hydrated hexaaluminium trichloride ($AlCl_3$, 6H2O) was
112 added. After 6 minutes, 1.6 L of 1 M NaOH was added. Absorbance was read immediately at 510

UNDER PEER REVIEW

113 nm against the control. A calibration curve was established with quercetin at different
114 concentrations between 0 and 1000 µg/mL under the same conditions as the samples. The results
115 obtained were expressed as mg quercetin equivalent (QE)/g dry extract.

116 **Total tannins content**

117 Proanthocyanidins (condensed tannins) were determined by the butanol/hydrochloric acid
118 (BuOH/HCl) method (Makkar et al., 1999). Briefly, 0.2 ml of an ammoniacal iron sulphate
119 solution (NH4Fe(SO4)2: 20 g/l) was added to a test tube, followed by 7 ml of BuOH/HCl (95 %
120 - 5 % ; v/v) and 0.2 mL of extract (30 mg/ml). The mixture was incubated in a water bath at 95°C
121 for 40 minutes. A pink or red colour was obtained. The proanthocyanidin concentrations of the
122 samples were determined by reading the optical density (OD) at a wavelength of 550 nm using a
123 METASH UV-visible spectrophotometer (UV-5200 PC). In order to derive the proanthocyanidin
124 content (Tp) of the samples, expressed as mg catechin equivalent (CE)/g dry extract, the
125 following formula was used for the calculations according to Aksamit-Stachurska et al. (2008):
126 Tp=DO/0.280 with OD = 0.280 corresponding to 1% catechin. Catechin was therefore used as
127 the standard molecule.

128 **Determination of antioxidant activity**

129 The protocol of Nair *et al*. (2007) is used is that study. To test tubes containing 3 mL of freshly
130 prepared FRAP solution (25 ml acetate buffer + 2.5 ml 10 mM Fe3+-TPTZ prepared in a 40 mM
131 HCl solution + 2.5 mL 20 mM FeCl3-6H2O), 10 µl of sample was added. The reading was taken
132 at 593 nm on a METASH UV-visible spectrophotometer (UV-5200 PC) against the blank after 10
133 min incubation. A calibration curve was constructed from a concentration range (0-1800 µM) of

UNDER PEER REVIEW

134 a solution of iron sulphate ($FeSO_4$, $7H_2O$) dissolved in methanol. The result was expressed as

135 µmol FeSO4 equivalent/mg dry extract.

136 **Antimicrobial activity**

137 **Preparation of hydroethanol extracts and antibiotics**

138 700 mg of hydroethanol extracts from the root and aerial parts were dissolved in 7 ml of

139 hydroethanol solution (v/v) and filtered once through a sterile 0.20 µm Millipore filter under

140 aseptic conditions. The extracts were then placed in sterile Falcon tubes and stored at 4°C for

141 future use. Chloramphenicol 1 mg/ml (1%) and griseofulvin 20 µmg/ml were prepared as

142 reference antibiotics for the bacteria and fungi tested.

143 **Preparation of inocula**

144 Bacterial inocula were prepared from Müller-Hinton broth and incubated for 24 hours. Bacterial

145 suspensions with a turbidity of 0.5 Mac Farland (4.10^8 CFU/ml) were prepared using sterile

146 physiological water (NaCl; 0.9 %). Conidial suspensions of *Trichophyton tonsurans* and

147 *Trichophyton mentagrophytes* were prepared from 7-day-old cultures on PDA agar incubated at

148 25°C. Conidia were harvested with sterile physiological water (NaCl; 0.9 %). Appropriate

149 dilutions were made from these conidial suspensions to prepare 0.5 Mac Farland turbidity fungal

150 inocula.

151 **Antimicrobial activity**

152 Minimum inhibitory concentrations (MICs), minimum bactericidal concentrations (MBCs) and

153 minimum fungicidal concentrations (MFCs) were determined using the microdilution method

154 described by Noumedem *et al*. (2013) and Anani *et al*. (2015) with some modifications. For each

UNDER PEER REVIEW

155 experiment, a sterility control (peptone broth and extract), a negative control (inoculum and
156 antibiotic) and a positive control (inoculum and peptone broth) were included. A 96-well plate
157 was used for the test and 100 µl of peptone broth was added to each well. Next, 100 µl of 200
158 mg/mL extract was added to the peptone broth in each well, followed by 100 µl of inoculum.
159 This second order regressive dilution gave final concentrations of 50, 25, 12.5, 6.25 and 3.125
160 µg/ml. Bacteria were incubated at 37°C for 24 h and at 25°C for 7 days for dermatophytes. The
161 MIC was defined as the lowest extract concentration at which the tested fungal strain showed no
162 visible growth. Minimum microbiocidal concentrations (MMC) were determined by plating 10
163 µl aliquots from wells showing no microbial growth on Mueller-Hinton or PDA agar and
164 incubating at 37°C for 24 hours for bacteria and at 25°C for 7 days for dermatophytes. The
165 antibiotic potency (AP) is considered microbiocidal if MFC/MIC ≤ 1, microbiostatic if
166 MFC/MIC > 1. The test has been repeated twice under aseptic conditions.

167 **Statistical analysis**

168 All data were integrated in an Excel™ 2016 spreadsheet, and processed with Graphpad Prism™
169 8.4.3 for statistical analysis.

170

UNDER PEER REVIEW

RESULTS

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 (Sodjinou et al., 2021). One of these famous species newly introduced in the Togolese flora, is *Chromolaena odorata* (L.) R. M. King & Robinson (Akpagana et al., 1993). 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 (Figure 1).

The climate is subequatorial Guinean type with two rainy seasons, and precipitation amounts varying between 700 and 1000 mm per year (Figure 2). Relative humidity is constantly high (> 50%) throughout the year and temperatures vary from 25° to 35°C (Anonyme, 1981).

UNDER PEER REVIEW

191

192

193

194

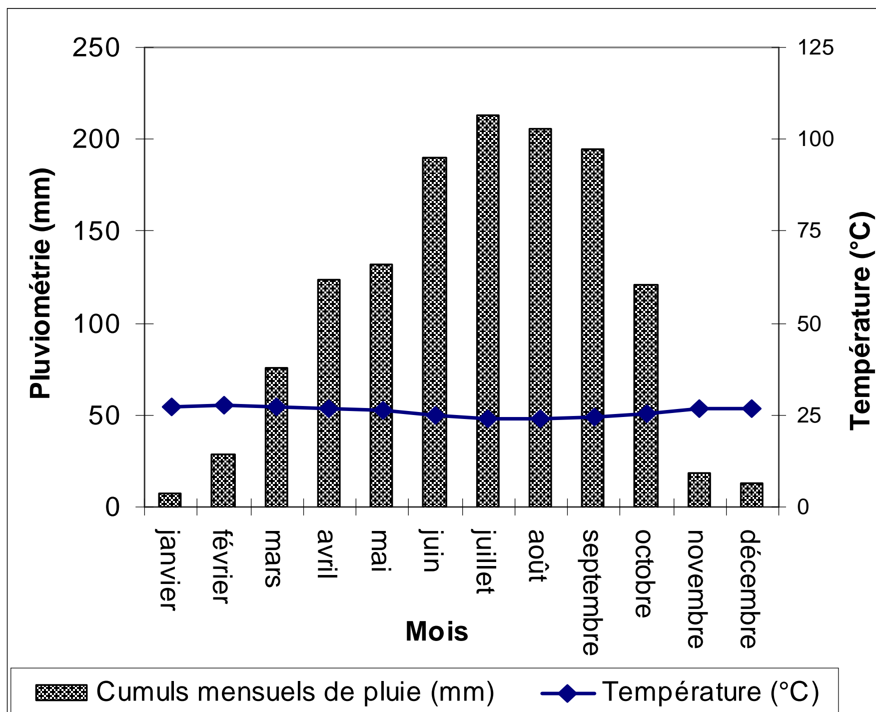
195

196

197

198

199



200 **Figure 1**. Ombrothermal curve of Lomé climatic Station (1971-2018)

201

202

203

204

205

206

207

208

UNDER PEER REVIEW

209

210

211

212

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

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

225 According to https://idao.cirad.fr/content/adventoi/especes/a/accin/accin_fr.html (accessed in 10
226 November 2023), *A. indica* is an erect semi-woody plant, reaching 1.5 m in height. It has a
227 taproot and its stem is ribbed, branching near the base. The leaves, light green, are simple,
228 alternate, long-petiolate; they are broadly oval, 1.2 cm to 6.5 cm × 1 cm to 4 cm. The base of the
229 leaves is rounded to briefly attenuate. The leaf margin is crenulated-serrated with an acute or
230 obtuse apex. The petiole is 1.5 to 5.5 cm long. It has catkin-shaped inflorescences with cup-

UNDER PEER REVIEW

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 (Figure 1). The pollen is roughly rounded and measures 10 to 12 µ in diameter. The fruit is a capsule, often decorated with excrescences, with three (3) cells, each containing a seed.

Figure 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)

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.

243 In West Africa, (Hutchinson & Dalziel, 1958) cited fourteen (14) species of *Acalypha* among
244 which four (4) are (*A. ciliata* Forssk., *A. crenata* Hochst. ex A. Rich., *A. racemosa* Wall. ex Baill.
245 et *A. segetalia* Müll. Arg.) are inventoried in Togo (Brunel et al., 1984).

246 Many uses in traditional medicine have been reported in Madagascar, Mauritius, Seychelles,
247 Reunion and East Africa where the plant species is intervenes in the treatment of skin and
248 respiratory ailments. Root decoction is taken against asthma and stomach aches
249 (https://www.pharmacopoeia.com/). However, as with any other species of the Euphorbiaceae
250 family, it is advisable to be very careful and avoid any hazardous use (Durasnel et al., 2018;
251 Giday & Teklehaymanot, 2013; Hassan-Abdallah et al., 2013; Schmitt et al., 2023;
252 Teklehaymanot, 2017). This herb is held in very high esteem in traditional Tamil Siddha
253 medicine, as it is believed to rejuvenate the skin.

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

259 **Phytochemical constituents**

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

UNDER PEER REVIEW

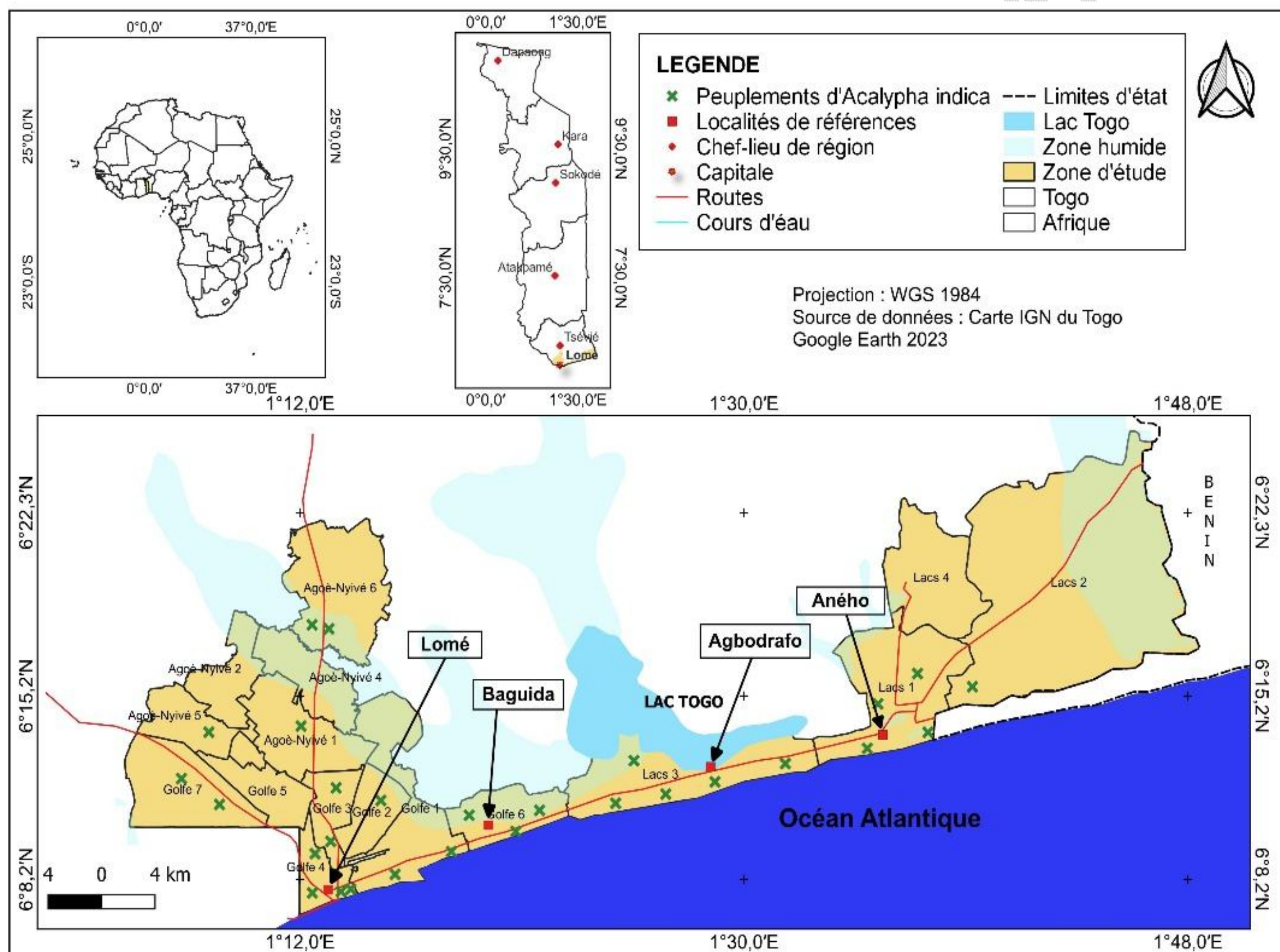
265

266

267

268

269



270 271 272 273 274 275 276 277 278 279

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

281 **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

282

283 **Total polyphenols, flavonoids and tannins content**

284 The contents of total polyphenols, total flavonoids and total tannins (catechin and gallic) in the
285 hydroethanolic extracts of the leafy stem and root of *A. indica* are shown in Table 2. Analysis of
286 the data showed that the leafy stem contained 94.8 mg GA of total polyphenols, 271.01 mg QE
287 of total flavonoids and 37.15 mg CE of total tannins per g of sample dry hydroethanolic extract.
288 In addition, the hydroethanol extract of the root contained 61.39 mg GAE of total polyphenols,
289 149.15 mg QE of total flavonoids and 23.62 mg CE of total tannins. In a comparative approach,
290 the contents of total polyphenols, total flavonoids and total tannins in the leafy stem of *A. indica*
291 L. were each higher than those measured in the root.

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

Molecules sought	Concentration/g Dried leafy stem	Concentration/g Dried root hydroethanol extract

UNDER PEER REVIEW

	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

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 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

Antimicrobial activity

Antimicrobial tests were carried out to determine the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), minimum fungicidal concentration (MFC) and antibiotic potency (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

UNDER PEER REVIEW

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.

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.

DISCUSSION

UNDER PEER REVIEW

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*. (2019) 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*. (2012) 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*. (2014), 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*. (2010), 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

UNDER PEER REVIEW

343 that the hydroethanolic extracts of the leafy stem and root of *A. indica* contained more total
344 flavonoids, followed by total polyphenols and total tannins. The low levels of bioactive
345 molecules in the roots compared to the leafy stem of the plant could be explained by the
346 anatomical structure of the roots, stem and leaves of the plant. Indeed, the aerial organs of plants
347 are rich in chlorophyll parenchyma, which contains epidermal cells (secretory tissues) capable of
348 producing and accumulating secondary metabolites in their cytoplasm. This would explain the
349 high levels of bioactive molecules in the leaf stem. Secretory tissues are not very abundant in
350 plant roots in favour of reserve cells. The contents obtained in this study are higher than those
351 reported by Godipurge *et al*. (2014). These authors reported that the highest contents of phenolic
352 compounds and total flavonoids were 9.27 mg TA/g and 8.75 mg Ru/g, respectively, in the
353 polyphenolic fraction of the ethanolic extract of *A. indica*. Kusrini *et al*. (2019) also reported that
354 the total phenolic content in the ethanolic extract of *A. indica* L. was 203.07 GAE/g. This
355 variation in the content of biomolecules in the extracts may be related to the biophysical factors
356 of where the plant samples were collected, the extraction solvents and the extraction method.
357 Phenolic compounds and flavonoids are important components of the plant and some of their
358 pharmacological effects could be attributed to the presence of these phytoconstituents
359 (Godipurge et al., 2014). Potential biological properties such as antibacterial, cytotoxic,
360 antimutagenic, antitumour, antidiabetic, antitumour, antiviral, antibacterial and antifungal
361 properties have been associated with the various compounds of *A. indica* extracts
362 (Chaichoowong et al., 2017).

363 The antioxidant properties of natural substances can be assessed using various methods,
364 including the FRAP test (Kpètèhoto et al., 2019). Analysis of the results of the present study
365 revealed that the hydroethanol extract of leafy stems showed the best antiradical activity.

UNDER PEER REVIEW

366 According to Kpètèhoto *et al.* (2019), total tannins, total flavonoids and total polyphenols
367 contained in the extracts are molecules with antibacterial and antioxidant activities. Furthermore,
368 Kang *et al*. (2003) reported that the polar polyphenolic molecules contained in plant extracts
369 contribute to an increase in their anti-free radical activity. This observation corroborates the
370 results of this study, which showed that the total phenolic content of the root (61.39 mg GAE/g)
371 was lower than that of the leafy stem (94.8 mg GAE/g). This could therefore explain the high
372 antioxidant activity of the hydroethanol extract of *A. indica* leafy stems compared to that of the
373 roots. Previous studies have already investigated the anti-free radical activity of *A. indica*
374 extracts. Indeed, Marwah *et al.* (2007) reported an antiradical activity of 81.6 ± 0.4 % for the
375 hydroethanol extract of the whole plant using the DPPH method, after incubation for 15 minutes
376 at a concentration of 50 µg/ml. In addition, hexane, chloroform and methanol extracts also
377 showed significant antioxidant activity with IC50 of 6.19, 5.70 and 7.79 mg/ml respectively
378 using a DPPH radical reduction assay and with IC50 of 6.13, 6.31 and 6.37 mg/ml respectively
379 using the ABTS radical reduction assay (Sanseera et al., 2012). A good correlation (correlation
380 coefficient, r = 0.812) between antioxidant activity and total phenolic compounds in wound
381 healing plants including *A. indica* was also reported (Govindarajan et al., 2008).

382 The microbiostatic properties demonstrated in this study justify the use of *A. indica* in traditional
383 medicine. The antimicrobial activity of plant extracts is related to a synergistic effect between the
384 different chemical groups present in plants, i.e. alkaloids, tannins and flavonoids, all of which
385 have antimicrobial activity (Athamena et al., 2010; Gessler et al., 1994; Hiremath et al., 1993;
386 Toure & Oduola, 2004). The antimicrobial activity of *A. indica* leaves against (08) bacterial
387 strains including *S. aureus*, *K. pneumoniae* and *E. coli* were evaluated (Govindarajan et al.,
388 2008). The results showed that hexane, chloroform, ethyl acetate and methanol extracts had MIC

UNDER PEER REVIEW

389 values of 0.312, 0.625, 0.312 and 0.156 mg/mL against *S. aureus* respectively. In contrast, the
390 MIC values against *K. pneumoniae* and *E. coli* were > 5 mg/ml. The MICs of the hydroethanolic
391 extracts of the leafy stem and root of *A. indica* L. against *Trichophyton mentagrophytes* and
392 *Trichophyton tonsurans* are very high compared to those of Noumedem *et al*. (2013), who
393 reported that the methanolic extract of *A. manniana* leaves had antifungal activity against *T.*
394 *mentagrophytes*, *T. equinum* and *T. terrestre* with MIC values of 0.25, 0.25 and 0.51 mg/mL,
395 respectively. This could be explained by the biomolecular content of each type of extract and the
396 sensitivity of the strains tested.

397

398 **CONCLUSION**

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

408

409

410

UNDER PEER REVIEW

411

412 **REFERENCES**

413 Akpagana, K., Guelly, K. A., & Gumedzoe, Y. M. (1993). A weed in the process of invading the Togolese
414 territory: Eupatorium odoratum L. [syn. Chromolaena odorata (L.) R.M. King & Robinson] (
415 Compositae). Acta Botanica Gallica, 140(5), 535–543.
416 https://doi.org/10.1080/12538078.1993.10515630

417 Aksamit-Stachurska, A., Korobczak-Sosna, A., Kulma, A., & Szopa, J. (2008). Glycosyltransferase
418 efficiently controls phenylpropanoid pathway. BMC Biotechnology, 8(1), 25.
419 https://doi.org/10.1186/1472-6750-8-25

420 Anani, K., Adjrah, Y., Ameyapoh, Y., Karou, S. D., Agbonon, A., de Souza, C., & Gbeassor, M. (2015).
421 Effects of hydroethanolic extracts of Balanites aegyptiaca (L.) Delile (Balanitaceae) on some
422 resistant pathogenic bacteria isolated from wounds. Journal of Ethnopharmacology, 164, 16–
423 21.

424 Anonymous. (nineteen eighty one). Atlas of Togo. Under the direction of Gù-Konu Y.E., Jeune Afrique
425 editions, 81 p.

426 Athamena, S., Chalghem, I., Kassah-Laouar, A., Laroui, S., & Khebri, S. (2010). Antioxidant and
427 antimicrobial activity of Cuminum cyminum L extracts. Lebanese Science Journal, 11(1), 69–81.

428 Balenghien, T., Vazeille, M., Reiter, P., Schaffner, F., Zeller, H., & Bicout, D. J. (2007). Evidence of
429 laboratory vector competence of Culex modestus for West Nile virus. Journal of the American
430 Mosquito Control Association, 23(2), 233–236.

431 Brunel, J.-F., Hiepko, P., & Scholz, H. (1984). Analytical flora of Togo: Phanerogams. Englera, 3–751.

UNDER PEER REVIEW

432 Chaichoowong, S., Bol, J.B., Bol, P., Gamse, T., & Sriariyanun, M. (2017). Chemical profiling of Acalypha
433 indica obtained from supercritical carbon dioxide extraction and Soxhlet extraction methods.
434 Orient J Chem, 33(1), 66–73.
435 Dineshkumar, B., Vigneshkumar, P., Bhuvaneshwaran, S. P., & Mitra, A. (2010). Phyto-pharmacology of
436 Acalypha indica: A Review. International Journal of Biosciences, Alternative and Holistic
437 Medicine, 1(2), 27.
438 Durasnel, P., Tantet, C., Chamouine, A., & Blondé, R. (2018). Traditional phytotherapy with Acalypha
439 indica inducing a hemolytic accident in patients with G6PD deficiency: A common
440 circumstance in Mayotte? Bulletin of the Exotic Pathology Society, 111(2), 81.
441 https://doi.org/10.3166/bspe-2018-0020
442 Elzagheid, M. I. (2018). Laboratory activities to introduce carbohydrates qualitative analysis to college
443 students. World J Chem Educ, 6(2), 82–86.
444 Fialkowski, M., Bishop, K. J. M., Chubukov, V. A., Campbell, C. J., & Grzybowski, B. A. (2005).
445 Architecture and Evolution of Organic Chemistry. Angewandte Chemie International Edition,
446 44(44), 7263–7269. https://doi.org/10.1002/anie.200502272
447 Françoise, A. A., Koffi, K., William, D., Emmanuel, B., Atèhèzi, T., Kosi, N. M., Kudzo, G. A., Jacques, D.,
448 Amégnona, A., & Sodokè, T. K. (2018). Ethnobotanical investigation into the traditional
449 management of female infertility in the savannah health region of Togo. European Scientific
450 Journal, 14. https://www.researchgate.net/profile/Kosi-Novidzro-
451 2/publication/322945517_Enquete_Ethnobotanique_Sur_La_Prise_En_Charge_Traditionnelle_
452 De_L'infertilite_Feminine_Dans_La_Region_Sanitaire_Des_Savanes_Au_Togo/links/600887212
453 99bf14088ad9dc9/Ethnobotanical-Investigation-On-Traditional-Care- Of-Female-Infertility-In-
454 The-Health-Region-Des-Savanes-Au-Togo.pdf

UNDER PEER REVIEW

455 Gessler, M. C., Nkunya, M. H., Mwasumbi, L. B., Heinrich, M., & Tanner, M. (1994). Screening Tanzanian
456 medicinal plants for antimalarial activity. Acta Tropica, 56(1), 65–77.
457 Giday, M., & Teklehaymanot, T. (2013). Ethnobotanical study of plants used in management of
458 livestock health problems by Afar people of Ada'ar District, Afar Regional State, Ethiopia.
459 Journal of Ethnobiology and Ethnomedicine, 9(1), 8. https://doi.org/10.1186/1746-4269-9-8
460 Godipurge, S.S., Biradar, J.S., & Mahurkar, N. (2014). Phytochemical and pharmacological evaluation of
461 Acalypha indica Linn in experimental animal models. International Journal of Pharmacognosy
462 and Phytochemical Research, 6(4), 973–979.
463 Govindarajan, M., Jebanesan, A., Reetha, D., Amsath, R., Pushpanathan, T., & Samidurai, K. (2008).
464 Antibacterial activity of Acalypha indica L. Eur Rev Med Pharmacol Sci, 12(5), 299–302.
465 Gupta, A. K., & Tandon, N. (2004). Reviews on Indian medicinal plants.
466 Hassan-Abdallah, A., Merito, A., Hassan, S., Aboubaker, D., Djama, M., Asfaw, Z., & Kelbessa, E. (2013).
467 Medicinal plants and their uses by the people in the Region of Randa, Djibouti. Journal of
468 Ethnopharmacology, 148(2), 701–713.
469 Hiremath, S. P., Badami, S., Swamy, H. K. S., & Biradar, J. S. (1993). Antimicrobial activity of various
470 extracts of Acalypha indica (Euphorbiaceae). Indian Journal of Microbiology, 33, 75–75.
471 Hutchinson, J., & Dalziel, J. M. (1958). Flora of west tropical Africa, Vol 1, crown agents for oversea
472 governments and administrations. Millbank, London, UK, 408–410.
473 Kang, D. G., keun Yun, C., & Lee, H. S. (2003). Screening and comparison of antioxidant activity of
474 solvent extracts of herbal medicines used in Korea. Journal of Ethnopharmacology, 87(2–3),
475 231–236.
476 Khaldi, A., Meddah, B., Moussaoui, A., Benmehdi, H., & Gouri, S. (2012). Phytochemical screening and
477 antifungal effect of certain plant extracts on the in vitro development of molds. European
478 Journal of Scientific Research, 80(3), 311–321.

UNDER PEER REVIEW

479 Khitri, W., Lachgueur, N., Tasfaout, A., Lardjam, A., & Khalfa, A. (2016). Antilithiasis plants used in
480 traditional medicine in the city of Oran, Algeria. Ethnobotanical and phytochemical approach.
481 Journal of Ethnoecology, 9. https://journals.openedition.org/ethnoecologie/2511
482 Kpètèhoto, H. W., Amoussa, A. M. O., Johnson, R. C., Houéto, E. E. M., Mignanwandé, F. M. Z.,
483 Yédomonhan, H., Loko, F., Bankolé, H., & Lagnika, L. (2019). Phytochemical analysis and
484 antioxidant potential of Ocimum gratissimum Linn (Lamiaceae) commonly consumed in the
485 Republic of Benin. Journal of Applied Biology and Biotechnology, 7(4), 75–83.
486 Kusrini, D., Fachriyah, E., & Prinanda, G. R. (2019). Isolation of phenolic acid in Acalypha indica l plants
487 and test total phenol also antioxidant test using DPPH method. IOP Conference Series:
488 Materials Science and Engineering, 509(1), 012033.
489 https://iopscience.iop.org/article/10.1088/1757-899X/509/1/012033/meta
490 Lipkus, A. H., Yuan, Q., Lucas, K. A., Funk, S. A., Bartelt, W. F., Schenck, R. J., & Trippe, A. J. (2008).
491 Structural Diversity of Organic Chemistry. A Scaffold Analysis of the CAS Registry. The Journal
492 of Organic Chemistry, 73(12), 4443–4451. https://doi.org/10.1021/jo8001276
493 Makkar, H. P., Gamble, G., & Becker, K. (1999). Limitation of the butanol–hydrochloric acid–iron assay
494 for bound condensed tannins. Food Chemistry, 66(1), 129–133.
495 Marwah, R. G., Fatope, M. O., Al Mahrooqi, R., Varma, G. B., Al Abadi, H., & Al-Burtamani, S. K. S.
496 (2007). Antioxidant capacity of some edible and wound healing plants in Oman. Food
497 Chemistry, 101(2), 465–470.
498 Mohammedi, Z. (2006). Study of the antimicrobial and antioxidant power of essential oils and
499 flavonoids of some plants from the Tlemcen region. Magister's memory. Abou Bakr Belkaïd
500 Tlemcen University. 105p.

UNDER PEER REVIEW

501 Mohan, C., Dinakar, S., Anand, T., Elayaraja, R., & SathiyaPriya, B. (2012). Phytochemical, GC-MS
502 analysis and Antibacterial activity of a Medicinal Plant Acalypha indica. Int J Pharm Tech Res,
503 4(3), 1050–1054.
504 Nair, V. D. P., Dairam, A., Agbonon, A., Arnason, J. T., Foster, B. C., & Kanfer, I. (2007). Investigation of
505 the Antioxidant Activity of African Potato (Hypoxis hemerocallidea). Journal of Agricultural and
506 Food Chemistry, 55(5), 1707–1711. https://doi.org/10.1021/jf0619838
507 Noumedem, J. A., Tamokou, J. D. D., Teke, G. N., Momo, R. C., Kuete, V., & Kuiate, J. R. (2013).
508 Phytochemical analysis, antimicrobial and radical-scavenging properties of Acalypha manniana
509 leaves. SpringerPlus, 2(1), 503. https://doi.org/10.1186/2193-1801-2-503
510 Radji, R., Kokou, K., & Akpagana, K. (2010). Diagnostic study of Togolese ornamental flora.
511 International Journal of Biological and Chemical Sciences, 4(2).
512 https://www.ajol.info/index.php/ijbcs/article/view/58159
513 Sanseera, D., Niwatananun, W., Liawruangrath, B., Liawruangrath, S., Baramee, A., Trisuwan, K., &
514 Pyne, S. G. (2012). Antioxidant and anticancer activities from aerial parts of Acalypha indica
515 Linn. https://ro.uow.edu.au/smhpapers/88/
516 Schmitt, C., Le Flécher, A., Caujolle, M., Bragança, C., Moulut, C., Maillot, A., Von Fabeck, K., de Haro,
517 L., & Simon, N. (2023). Poisoning by Acalypha indica, regarding two cases occurring in the
518 overseas territories of the Indian Ocean. Analytical and Clinical Toxicology, 35(3), S104–S105.
519 https://doi.org/10.1016/j.toxac.2023.08.070
520 Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). [14] Analysis of total phenols and
521 other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In Methods in
522 enzymology (Vol. 299, pp. 152–178). Elsevier.
523 https://www.sciencedirect.com/science/article/pii/S0076687999990171
UNDER PEER REVIEW

524 Sodjinou, K. E., ADJOSSOU, K., ETSE, K. D., JOHNSON, B. N., KODA, K. D., Marie-Luce, A., QUASHIE, R.
525 A., & KOKOU, K. (2021). Synopsis of croton hirtus herb, a newly reported weed for the flora of
526 Togo. 38, 485–494.
527 Teklehaymanot, T. (2017). An ethnobotanical survey of medicinal and edible plants of Yalo Woreda in
528 Afar regional state, Ethiopia. Journal of Ethnobiology and Ethnomedicine, 13(1), 40.
529 https://doi.org/10.1186/s13002-017-0166-7
530 Toure, Y. T., & Oduola, A. (2004). Focus: Malaria. Nature Reviews Microbiology, 2(4), 276–277.
531 Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in
532 mulberry and their scavenging effects on superoxide radicals. Food Chemistry, 64(4), 555–559.

UNDER PEER REVIEW