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Original Article

Complex secondary metabolites from *Ludwigia leptocarpa* with potent antibacterial and antioxidant activities

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Conflict of Interest:

There is no conflict of interest to disclose.

Summary

Diarrhea continues to be one of the most common causes of morbidity and mortality among infants and children in developing countries. The present study aims to evaluate the antibacterial and antioxidant activities of extracts and compounds from *Ludwigia leptocarpa*, a plant traditionally used for its vermifugal, anti-dysenteric and antimicrobial properties. The methanol extract was prepared by maceration from dried whole plant and successively extracted with ethyl acetate and *n*-butanol to obtain EtOAc and *n*-BuOH extracts respectively. The column chromatography of the EtOAc and *n*-BuOH extracts followed by purification of different fractions led to the isolation of ten known compounds. Structures of isolated compounds were assigned on the basis of spectra analysis, and by comparison with those from the literature. The antioxidant activity was evaluated by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and gallic acid equivalent antioxidant capacity (GAEAC) assays. The antibacterial activity was assessed by performing minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) against the strains of Gram-positive bacterium, *Staphylococcus aureus* (a major cause of community and hospital-associated infection), and Gram-negative multi-drug resistant bacteria, *Vibrio cholerae* (causative agent of cholera) and *Shigella flexneri* (causative agent of shigellosis). All of the extracts showed different degrees of antioxidant and antibacterial activities. 2*β*-hydroxyoleanolic acid, (2*R*,3*S*,2''*S*)-3''',4',4''',5,5'',7,7''-heptahydroxy-3,8"-biflavanone and luteolin-8-*C*-glucoside displayed the largest antibacterial and antioxidant properties which were in some cases equal or higher than those of reference drugs. The overall results of the present study shows that *L. leptocarpa* has potentials as a source of natural anti-diarrhoeal and anti-free radical products, given further investigations.

***Keywords*:** *Ludwigia leptocarpa*, Onagraceae, triterpenoids, flavonoids, antibacterial, antioxidant

1. Introduction

In developing countries, particularly in Africa, poor sanitation exposed the people to a wider array of microbial pathogens which increases their susceptibility to bacterial infections (*1*). It is reported that each year, 300,0000 children die of diarrhoeal diseases. Among the diarrhoeal diseases, cholera is one of the most important causal elements in terms of severity of the disease and outcomes. Several epidemics of cholera have been reported from different parts of Cameroon and abroad (*2-5*). *Vibrio cholerae* strains belonging to O1 and O139 serogroups are responsible to cause cholera in the form of epidemics and pandemics (*6,7*). In recent years, reported cholera cases increased steadily reaching more than 300' 000 cases including more than 7'500 deaths during 2010 (*8*). As populations of poor countries continue to coalesce in mega-cities with low levels of sanitation and people move rapidly around the globe, new and more virulent strains of *V. cholerae* are expected to disseminate more rapidly (*9,10*). This makes cholera one of the most rapidly fatal infectious illnesses known.

The continuous emergence of multidrug-resistant (MDR) *Vibrio cholerae* strains drastically, reduces the efficacy of our antibiotic armory and, consequently, increases the frequency of therapeutic failure (*11,12*). In many regions, affected by this pathogen, local and indigenous plants are often the only available means of treating such infections. Among the known plant species on earth (estimated at 250,000–500,000) only a small fraction have been investigated for the presence of antimicrobial compounds and only 1–10% of plants are used by humans (*13,14*). Plant natural products can also have antioxidant potential. These include phenolic compounds, alkaloids, terpenoids and essential oils. Plant based antioxidant compounds (*15*) play a defensive role by preventing the generation of free radicals and hence are extremely beneficial to alleviate infectious diseases generating free radicals as well as diseases caused by oxidative stress such as cardiovascular diseases, diabetes, inflammation, degenerative diseases, cancer, anemia, and ischemia (*16*).

Ludwigia leptocarpa (Nutt) Hara (Onagraceae or Oenotheraceae) is a herbaceous plant species that is also well represented in North America and in tropical Africa (*17*). In traditional medicine in Nigeria, an infusion of the plant is part of a mixture to treat rheumatism (*18*). A leaf infusion has laxative, vermifugal and anti-dysenteric properties. Previous works on this genus have revealed the presence of flavonoids (*19,20*), cerebrosides and triterpenoids (*20,21*). It has recently been reported that alcoholic extracts of the leaves of *L. octovalvis, L. abyssinica* and *L. decurrens* have potential antioxidant, antibacterial and antifungal activities (*22,23*). To the best of our knowledge, there has been no documented report on the antioxidant and antibacterial properties of *L. leptocarpa* against diarrhea strains. Hence, the aim of this study was to investigate the antibacterial and antioxidant properties of extracts and compounds from *L. leptocarpa*.

2. Materials and methods

2.1. Experimental

The IR spectra were recorded with a Shimadzu FT-IR-8400S (Shimadzu, France) spectrophotometer. ^{1}H (500 MHz) and ^{13}C (125 MHz) Nuclear Magnetic Resonance (NMR) spectra were recorded on a BRUKER Avance DRX-500 spectrometer (Bruker, Wissembourg, France) equipped with a BBFO + 5 mm sonde. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectra were recorded on a BRUKER Avance III-600 spectrometer (Bruker, Wissembourg, France) equipped with a cryoplatform using CD_3OD with tetramethylsilane (TMS) as the internal standard. Time of flight electrospray ionization mass spectrometry (TOF-ESIMS) and High-resolution time of flight electrospray ionization mass spectrometry (HR-TOFESIMS) experiments were performed using a Micromass Q-TOF micro instrument (Manchester, UK) with an electrospray source. The samples were introduced by direct infusion in a solution of methanol (MeOH) at a rate of 5 μL min^{-1}.

Column chromatography was run on Merck silica gel (VWR, France) 60 (70-230 mesh) and gel permeation on Sephadex LH-20 (VWR, France), while thin layer chromatography (TLC) was carried out on silica gel GF254 pre-coated plates with detection accomplished by spraying with 50% H_2SO_4 followed by heating at 100 °C or by visualizing with a ultra-violet (UV) lamp at 254 and 365 nm.

2.2. Plant material

The whole plant of *L. leptocarpa* was collected in Foto village (Menoua Division, Western region of Cameroon), in April 2011. Authentication was performed by Victor Nana, a botanist of the Cameroon National Herbarium, Yaoundé, where a voucher specimen (N° 38782/HNC) has been deposited.

2.3. Extraction and isolation

The dried whole plant of *L. leptocarpa* (4 kg) was extracted with MeOH at room temperature for 3 days, and the extract was concentrated to dryness under reduced pressure to yield a dark crude extract (102 g). Part of residue obtained (97 g) was suspended in water (200 mL) and successively extracted with ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH) which were concentrated to dryness under reduced pressure to afford EtOAc (20 g) and *n*-BuOH (40 g) extracts, respectively.

According to the antimicrobial and antioxidant assays, the EtOAc and *n*-BuOH extracts were submitted to further separation and purification. Part of EtOAc extract (15 g) was purified over silica gel column eluted with hexane containing increasing EtOAc (10%, 20%, 30%, 40%, 50%, 60%, 70% and 80%) and with EtOAc containing increasing MeOH (10% and 20%). Six fractions were obtained A, B, C, D, E and F. Fraction D (1.7 g) was purified over silica gel column eluted with the mixture hexane-EtOAc (7:3) to give compounds **1** and

2 (17 mg and 22 mg, respectively). Fraction E (3.1 g) was purified over silica gel column chromatography eluted with the mixture hexane-EtOAc (6:4) to give compound **3** (17 mg). Part of *n*-BuOH extract (30 g) was purified over silica gel column chromatography, eluted with EtOAc containing increasing MeOH (10%, 20%, 30%, 40% and 50%). Five fractions (G_1-G_5) were obtained. Fraction G_1 (2.5 g) was purified over silica gel column eluted with EtOAc to give the compounds **4** (19 mg) and **5** (16 mg). Fraction G_2 (3.1 g) was purified over silica gel column chromatography eluted with the mixture EtOAc-MeOH (8.5:1.5) to give compounds **5** (25 mg) and **6** (13 mg). Fractions G_3 and G_4 (5.4 g) were combined and purified by silica gel column chromatography eluting with the mixture of EtOAc-MeOH-H_2O (8:1:1) to give the compounds **7** (38 mg) and **8** (24 mg). Fraction G_5 (2.5 g) was purified by silica gel column chromatography eluting with the mixture of EtOAc-MeOH-H_2O (7:2:1) to give the compounds **9** (66 mg) and **10** (40 mg).

Oleanolic acid (**1**): white amorphous powder from hexane-EtOAc; $C_{30}H_{48}O_3$.

2*β*-hydroxyoleanolic acid (**2**): white amorphous powder from hexane-EtOAc; $C_{30}H_{48}O_4$.

(2*R*,3*S*,2''*S*)-3''',4',4''',5,5'',7,7''-heptahydroxy-3,8"-biflavanone (**3**): white amorphous powder from hexane-EtOAc; $C_{30}H_{22}O_{11}$; high resolution electron impact mass spectrometry (HRESIMS, positive-ion mode) m/z: 581.1057 $[M + Na]^+$(calcd. for $C_{30}H_{22}O_{11}Na$:581.1060).

Ellagic acid (**4**): yellow powder from EtOAc; $C_{14}H_6O_8$.

β-sitosterol-3-*O*-*β*-D-glucopryranoside (**5**): white amorphous powder from EtOAc$C_{35}H_{60}O_6$.

Luteolin-8-*C*-glucoside (**6**): yellow amorphous powder from EtOAc$C_{21}H_{20}O_{11}$.

28-*O*-*β*-D-xylopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→2)-[*α*-L-arabinopyranosyl-(1→3)]-4-*O*-(3'-hydroxybutanoyloxy-3-hydroxybutanoyloxy)-*β*-D-fucopyranosyl zanhic acid (**7**): white amorphous solid from EtOAc; $C_{60}H_{94}O_{27}$; HRESIMS (positive-ion mode) m/z: 1269.5870 $[M + Na]^+$(calcd. for $C_{60}H_{94}O_{27}Na$: 1269.5880).

3-*O*-*β*-D-glucopyranosyl-28-*O*-*β*-D-xylopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→2)-4-*O*-(3'-hydroxybutanoyloxy-3-hydroxybutanoyloxy)-*β*-D-fucopyranosyl medicagenic acid (**8**): white amorphous solid from EtOAc; $C_{61}H_{96}O_{27}$; HRESIMS (positive-ion mode) m/z: 1283.6044 $[M + Na]^+$ (calcd. for $C_{61}H_{96}O_{27}Na$: 1283.6037).

3-*O*-*β*-D-glucopyranosyl-28-*O*-*β*-D-xylopyranosyl-(1→4)-*α*-L-rhamnopyranosyl(1→2)-[*α*-L-arabinopyranosyl-(→3)]-4-*O*-(3'-hydroxybutanoyloxy-3-hydroxybutanoyloxy)-*β*-D-fucopyranosyl zanhic acid (**9**): white amorphous solid from EtOAc; $C_{66}H_{104}O_{32}$; HRESIMS (positive-ion mode) m/z: 1431.6395$[M+Na]^+$(calcd. for $C_{66}H_{104}O_{32}Na$: 1431.6408).

3-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl-28-*O*-*β*-D-xylopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-(1→2)-[*α*-L- arabinopyranosyl-(1→3)]-4-*O*-(3'-hydroxybutanoyloxy-3-hydroxybutanoyloxy)-*β*-D-fucopyranosyl zanhic acid (**10**): white amorphous solid from EtOAc; $C_{72}H_{114}O_{37}$; HRESIMS (positive-ion mode) m/z: 1593.6927$[M + Na]^+$(calcd. for $C_{72}H_{114}O_{37}Na$: 1593.6937).

2.4. Antibacterial assay

2.4.1. Microorganisms

A total of six bacterial strains were tested for their susceptibility to compounds and these strains were taken from our laboratory collection (kindly provided by Dr. T. Ramamurthy, NICED, Kolkata). Among the clinical strains of *Vibrio cholerae* used in this study, strains NB2 and SG24(1) belonged to O1 and O139 serotypes, respectively. These strains were able to produce cholera toxin and hemolysin (*24*,*25*). The other strains used in this study were *V. cholerae* non-O1, non-O139 (strains CO6 and PC2) (*24*); and *Shigella flexneri* (*26*). The *V. cholerae* non-O1 and non-O139 strains, were positive for hemolysin production but negative for cholera toxin production (*24*). The American Type Culture Collection (ATCC) strain, *Staphylococcus aureus* ATCC 25923, was used for quality control.

The bacterial strains were maintained on agar slant at 4 °C and subcultured on a fresh appropriate agar plates 24 h prior to any antibacterial test. The Mueller Hinton Agar (MHA) was used for the activation of bacteria. The Mueller Hinton Broth (MHB) and nutrient agar (Hi-Media) were used for the MIC and MBC determinations respectively.

2.4.2. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

MIC and MBC of extracts/compounds were assessed using the broth microdilution method recommended by the National Committee for Clinical Laboratory Standards (*27*,*28*) with slight modifications. Each test sample was dissolved in dimethylsulfoxide (DMSO, Fisher chemicals) to give a stock solution. The 96-well round bottom sterile plates were prepared by dispensing 180 µl of the inoculated broth ($1x10^6$ CFU/mL) into each well. A 20 µL aliquot of the compounds was added. The concentrations of tested samples varied from 0.125 to 1024 µg/mL. The final concentration of DMSO in each well was <1% [preliminary analyses with 1% (v/v) DMSO did not inhibit the growth of the test organisms]. Dilutions of ampicillin (Sigma-Aldrich, Steinham, Germany) and tetracycline (Sigma-Aldrich, Steinham, Germany) served as positive controls, while broth with 20 µL of DMSO was used as negative control. Plates were covered and incubated for 24 h at 37 °C. After incubation, minimum inhibitory concentrations (MIC) were read visually; all wells were plated to nutrient agar (Conda, Madrid, Spain) and incubated for 24 h at 37 °C. The lowest concentrations that yielded no growth after this subculturing were taken as the minimum bactericidal concentration (MBC) values.

2.5. Antioxidant assay

2.5.1. DPPH free radical scavenging assay

The free radical scavenging activity of extracts as well as their isolated compounds was performed according to Brand-Williams et al. (*29*) with slight modifications. Briefly, different concentrations (10 to 2000 µg/mL) of extracts/compounds and vitamin C (Sigma-Aldrich, Steinham, Germany) were thoroughly mixed with 3 mL of methanolic DPPH solution (20 mg/L) in test-tubes and the resulting solution was kept standing for 30 minutes at room temperature before the optical density (OD) was measured at 517 nm. The measurement was repeated with three sets and an average of the reading was considered. The percentage radical scavenging activity was calculated from the following formula: % scavenging [DPPH] = $[(A_0 - A_1)/A_0] \times 100$. Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the samples. IC_{50} value was determined from the graph obtained using standard vitamin C by using the "y = mx + c" formula from the slope of the graph.

2.5.2. Gallic acid equivalent antioxidant capacity (GAEAC) assay

The GAEAC test was done as previously described (*30*) with slight modifications. In a quartz cuvette, to 950 µL acetate buffer (pH =5.0, 100 mM), the following were added: 20 µL laccase (1 mM stock solution), 20 µL test sample, 10 µL ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (74 mM stock solution). The laccase were purified from *Sclerotinia sclerotiorum* according to the protocol described (*31*). The sample concentrations in the assay mixture were 800, 400, 200, 100, 10 µg/mL for the extracts and 200, 100, 50, 25, 125.5 µg/mL for the isolated compounds. The content of the generated $ABTS^{\bullet+}$ radical was measured at 420 nm after 240 s reaction time and was converted to gallic acid equivalents by the use of a calibration curve (Pearson's correlation coefficient: $r = 0.996$) constructed with 0, 4, 10, 14, 28, 56, 84 µM gallic acid standards rather than Trolox. Experiments were done in triplicate.

234 *2.6. Statistical analysis*

235 Data were analyzed by one-way analysis of variance followed by Waller-Duncan Post
236 Hoc test. The experimental results were expressed as the mean ± Standard Deviation (SD).
237 Differences between groups were considered significant when p <0.05. All analyses were
238 performed using the Statistical Package for Social Sciences (SPSS, version 12.0) software.

239

240 **3. Results and discussion**

241 *3.1. Chemical analysis*

242 According to the antibacterial assays from MeOH, EtOAc and *n*-BuOH extracts, the
243 EtOAc and *n*-BuOH extracts were submitted to further separation and purification. This led to
244 the isolation of ten compounds. Structures (Figure 1) of these compounds have been assigned
245 on the basis of spectroscopic data (^{1}H and ^{13}C NMR, ^{1}H-^{1}H COSY, HSQC, HMBC, ROESY
246 and NOESY), mass spectrometry, and by comparison to their data with those of the literature.
247 Hence, the isolated compounds were identified as oleanolic acid (**1**) (*32*); 2*β*-
248 hydroxyoleanolic acid (**2**) (*32*); (2*R*,3*S*,2''*S*)-3''',4',4''',5,5'',7,7''-heptahydroxy-3,8"-
249 biflavanone (**3**) (*33*); ellagic acid (**4**) (*34*); 3-*O*-*β*-D-glucopryranosyl-*β*- sitosterol (**5**) (*35*);
250 luteolin-8-*C*-glucoside (**6**) (*36*); 28-*O*-*β*-D-xylopyranosyl-(1→4)-*α*-L-rhamnopyranosyl-
251 (1→2)-[*α*-L-arabinopyranosyl-(1→3)]-4-*O*-(3'-hydroxybutanoyloxy-3-hydroxybutanoyloxy)-
252 *β*-D-fucopyranosyl zanhic acid (**7**) (*21*); 3-*O*-*β*-D-glucopyranosyl-28-*O*-*β*-D-xylopyranosyl-
253 (1→4)-*α*-L-rhamnopyranosyl-(1→2)-4-*O*-(3'-hydroxybutanoyloxy-3-hydroxybutanoyloxy)-
254 *β*-D-fucopyranosyl medicagenic acid (**8**) (*21*); 3-*O*-*β*-D-glucopyranosyl-28-*O*-*β*-D-
255 xylopyranosyl-(1→4)-*α*-L-rhamnopyranosyl(1→2)-[*α*-L-arabinopyranosyl-(→3)]-4-*O*-(3'-
256 hydroxybutanoyloxy-3-hydroxybutanoyloxy)-*β*-D-fucopyranosyl zanhic acid (**9**) (*20*); 3-*O*-
257 *β*-D-glucopyranosyl-(1→4)-*β*-D-glucopyranosyl-28-*O*-*β*-D-xylopyranosyl-(1→4)-*α*-L-

258 rhamnopyranosyl-(1→2)-[*α*-L- arabinopyranosyl-(1→3)]-4-*O*-(3'-hydroxybutanoyloxy-3-
259 hydroxybutanoyloxy)-*β*-D-fucopyranosyl zanhic acid (**10**) (*21*).
260
261 *3.2. Antibacterial activity*
262 The susceptibility pattern and inhibition parameters of the tested organisms to the
263 extracts and isolated compounds are indicated below (Table 1). The wells containing a
264 concentration of 64-512 μg/mL of MeOH, EtOAc and *n*-BuOH extracts inhibited the visible
265 growth of all the bacterial species. The most sensitive bacterial species were *S. aureus* and *S.*
266 *flexneri*, while *V. cholerae* SG24 (1) and *V. cholerae* NB2 were the most resistant species to
267 tested samples. All the three plant extracts showed less antibacterial activity when
268 compared with tetracycline. However, these extracts were active against *V. cholerae* NB2, *V.*
269 *cholerae* PC2 and *S. flexneri* which were not sensitive to ampicillin. The antimicrobial
270 activity of plant extract was considered to be good if its MIC was less than 100.0 μg/mL,
271 moderate if MIC was from 100.0 to 500.0 μg/mL and poor over 500.0 μg/mL (*37*). Hence, the
272 MeOH, EtOAc and *n*-BuOH extracts of *L. leptocarpa* exhibited good activity at a MIC value
273 of 64 μg/mL against *S. aureus* whereas only the MeOH extract displayed poor activity against
274 *V. cholerae* SG24 (1). The results of the *L. leptocarpa* extracts showed that this plant species
275 is potential source of antibacterial agents. This *in vitro* study corroborated the previous
276 antibacterial activities of alcoholic extracts from *L. octovalvis, L. abyssinica* and *L. decurrens*
277 leaves against *Staphylococcus aureus* (*22,23,38*).
278 The lowest MIC and MBC values of 2 μg/mL were recorded on *S. aureus* with
279 compound **3**; highlighting its good antibacterial potential, as the activity on *S. aureus* was
280 higher than that of ampicillin (MIC = 16 μg/mL and MBC = 16 μg/mL) and tetracycline (MIC
281 = 16 μg/mL and MBC = 128 μg/mL) used as reference antibacterial drugs. However, the
282 highest MIC value of 512 μg/mL was recorded on *V. cholerae* SG24 (1) with MeOH extract,

283 and the highest MBC value of 512 µg/mL was obtained on *V. cholerae* SG24 (1), *V. cholerae*
284 CO6 and *V. cholerae* PC2 with the MeOH extract. A lower MBC/MIC (≤4) value signifies
285 that a minimum amount of plant extract/ isolated compound is used to kill the bacterial
286 species, whereas, a higher value signifies the use of comparatively higher concentration of the
287 compounds is needed for the control of the microorganism (*39*).

288 The antibacterial activities of isolated compounds from *L. leptocarpa* are in the order
289 as compound **3** > compound **6** > compound **2** > compound **4** > compounds **8**, **9** > compound
290 **10** > compound **7** > compound **1**. Compounds **3, 6, 2, 4, 8, 9** and **10** were active against all the
291 tested pathogens whereas compound **1** was active only on *S. flexneri* and *S. aureus*. No
292 activity was noted on compound **5** (result not shown). Antimicrobial cut-off points have been
293 defined by several authors to enable the understanding of the potential of pure compounds as
294 follows: significant activity (MIC < 10 µg/mL), moderate activity (10 < MIC ≤ 100 µg/mL),
295 and low activity (MIC> 100 µg/mL) (*40,41*). Based on this, the antibacterial activity of
296 compound **3** on *V. cholerae* CO6, *V. cholerae* NB2, *V. cholerae* PC2, *S. flexneri* and *S. aureus*
297 as well as that of compound **6** on *Shigella flexneri* SDINT and *Staphylococcus aureus* ATCC
298 25923 can be considered significant. The strains of *V. cholerae* NB2, PC2 (*24,25*) and
299 *Shigella flexneri* (*26*) included in the present study were MDR clinical isolates and these were
300 resistant to commonly used drugs such as ampicillin, streptomycin, tetracycline, nalidixic
301 acid, furazolidone, *co*-trimoxazole, etc. However, most of the tested samples displayed
302 antibacterial activities against these microbial strains; suggesting that their administration may
303 represent an alternative treatment against the *V. cholerae*, the causative agent of dreadful
304 disease cholera and *S. flexneri*, the causative agent of shigellosis. Taking into account the
305 medical importance of the tested bacteria, this result can be considered as promising in the
306 perspective of new antibacterial drugs development. The antibacterial activities of oleanolic
307 acid, ellagic acid and 2*β*-hydroxyoleanolic acid corroborate those of the early reports (*42,43*).

All the compounds found to be active in the present study belong to the triterpenoid, flavonoid and phenolic acid groups. Although triterpenoid, flavonoid and phenolic acid compounds have been reported to possess antibacterial activity (*39,44*), no study has been reported on the activity of compounds **3**, **6**-**10** against these types of MDR pathogenic bacterial strains.

The mechanism of action of terpenoids (**1**, **2**, **5**, **7**-**10**) is not fully understood, but is speculated to involve membrane disruption by the lipophilic compounds (*45*). The inhibition of tested bacterial strains by phenolic acid (**4**) may be due to iron deprivation or hydrogen bounding with vital proteins such as microbial enzymes (*46*). The mechanism of the active flavonoids (**3, 6**) is still to be studied; nevertheless, their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. More lipophilic flavonoids may also disrupt microbial membranes (*47*).

3.3. Antioxidant activity

The MeOH, EtOAc and *n*-BuOH extracts and their isolated compounds were subjected for the evaluation of antioxidant activity by using two *in vitro* model systems. The results were expressed as gallic acid equivalent antioxidant capacity of tested samples (Figure 2) and as equivalent concentrations of test samples scavenging 50% of DPPH radical (Figure 3). $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ radical scavenging activities were observed in all the extracts. The MeOH and EtOAc extracts showed dominant activity followed by *n*-BuOH extract (Figures 2 and 3) among the extracts. The results indicate the potential of the tried extracts as a source of natural antioxidants with potential application to reduce oxidative stress with consequent health benefits. The antioxidant capacity of tried extracts may be due to the hydrogen donating ability of phenols and flavonoids present in them. Similarly, early reports have shown phenolic compounds to contribute significantly to the antioxidant activity of medicinal

plants (*39*,*48*). The compounds, which showed the strongest DPPH· and ABTS·+ radical scavenging activities, are compounds **2** (EC_{50} = 7.66 µg/mL; GAEAC= 71.64 µg/mL), **3** (EC_{50} = 1.09 µg/mL; GAEAC= 96.88 µg/mL) and **6** (EC_{50} = 10.34 µg/mL; GAEAC= 67.35 µg/mL), while the others (compounds **4** and **8**) show moderate antioxidant properties. Compounds **1**, **5**, **7, 9** and **10** were found not active in both the two model systems. Compound **3** was the most antioxidant compound and its DPPH· radical scavenging activity was equal to that of vitamin C used in the present study as reference antioxidant drug. The above finding suggests that compound **3** is the best candidate to combat diseases associated with oxidative stress. This is very promising in the perspective of antioxidant drug discover from plant origin. The antioxidant activities of compounds **2** and **4** are in agreement with those of the literature (*42*,*49*). However, the antioxidant activity of the MeOH, EtOAc and *n*-BuOH extracts from *L. leptocarpa* as well as that of compounds **3**, **6** and **8** are presented here for the first time.

4. Conclusion

The results show that the MeOH and EtOAc extracts from *L. leptocarpa* as well as compounds **2**, **3** and **6** possess the largest antibacterial and antioxidant properties and thus the plant has potentials as a source of natural health-giving products, given further investigations.

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480

481 **Table 1. Antibacterial activity (MIC and MBC in µg/ml) of extracts, isolated compounds**

482 **and reference antibacterial drugs**

Extracts/ compounds	Inhibition parameters	*Vibrio cholerae* SG24 (1)	*Vibrio cholerae* CO6	*Vibrio cholerae* NB2	*Vibrio cholerae* PC2	*Shigella flexneri* SDINT	*Staphylococcus aureus ATCC 25923*
MeOH extract	MIC	512	256	256	256	128	64
	MBC	512	512	256	512	128	128
	MBC/MIC	1	2	1	2	1	2
EtOAc extract	MIC	128	256	128	128	128	64
	MBC	256	256	>512	256	128	128
	MBC/MIC	2	1	/	2	1	2
n-BuOH extract	MIC	256	256	128	256	128	64
	MBC	256	>512	256	256	128	128
	MBC/MIC	1	/	2	1	1	2
1	MIC	>256	>256	>256	>256	256	256
	MBC	/	/	/	/	>256	>256
	MBC/MIC	/	/	/	/	/	/
2	MIC	128	64	64	64	16	16
	MBC	128	64	64	128	32	16
	MBC/MIC	1	1	1	2	2	1
3	MIC	16	8	8	8	4	2
	MBC	16	8	8	8	4	2
	MBC/MIC	1	1	1	1	1	1
4	MIC	128	64	64	128	64	32
	MBC	>256	128	64	256	64	32
	MBC/MIC	/	2	1	2	1	1
6	MIC	16	32	32	16	4	4
	MBC	32	32	32	16	8	8
	MBC/MIC	2	1	1	1	2	2
7	MIC	>256	256	256	256	128	128
	MBC	/	>256	>256	>256	>256	128
	MBC/MIC	/	/	/	/	/	1
8	MIC	128	256	128	128	128	64
	MBC	>512	256	256	256	128	64
	MBC/MIC	/	1	2	2	1	1
9	MIC	256	128	128	128	64	64
	MBC	>256	256	128	256	128	64

	MBC/MIC	/	2	1	2	2	1
10	MIC	256	256	256	256	128	128
	MBC	>256	>256	>256	>256	>256	128
	MBC/MIC	/	/	/	/	/	1
Ampicillin	MIC	16	16	>512	>512	>512	4
	MBC	16	16	>512	>512	>512	4
	MBC/MIC	1	1	/	/	/	1
Tetracycline	MIC	0.5	2	0.5	0.5	16	2
	MBC	4	16	4	4	128	8
	MBC/MIC	8	8	8	8	8	4

/: not determined; MIC: Minimum Inhibitory Concentration; MBC Minimum Bactericidal Concentration.

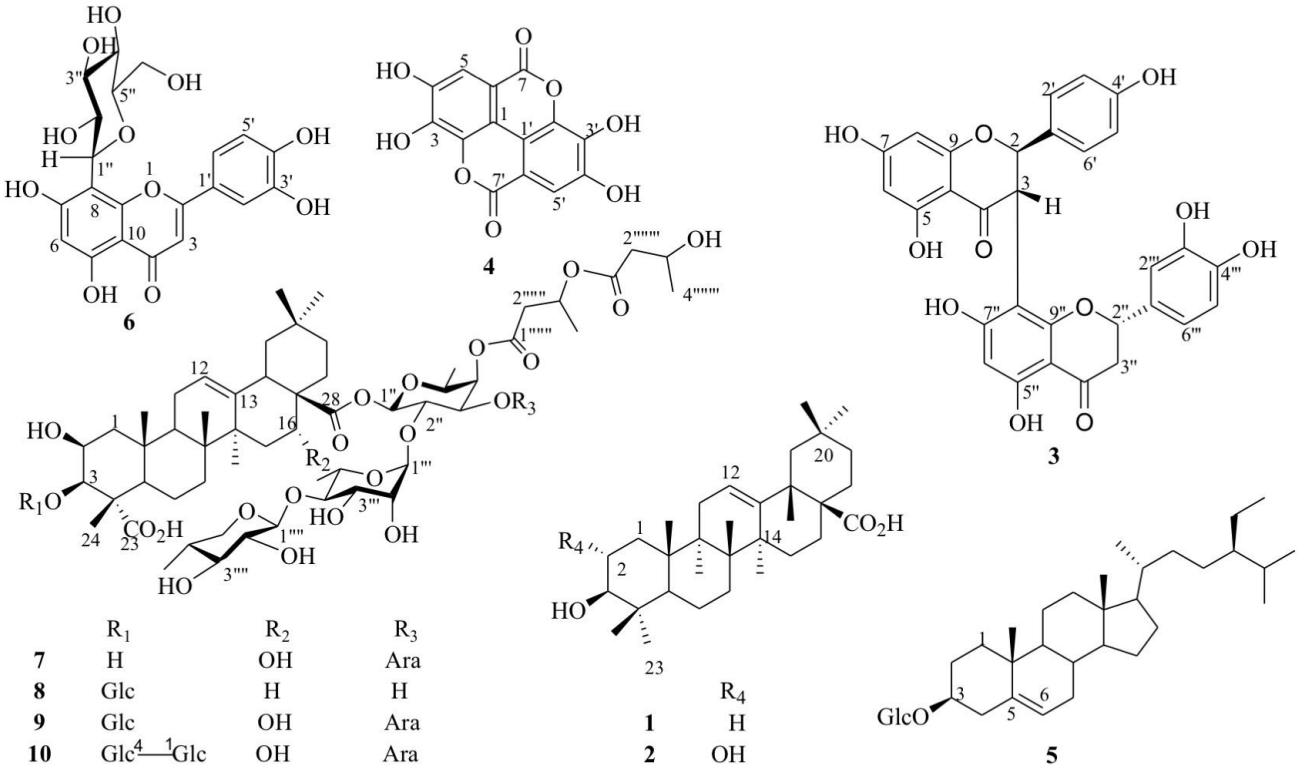


Figure 1. Structures of compounds isolated from the whole plant of *L. leptocarpa*

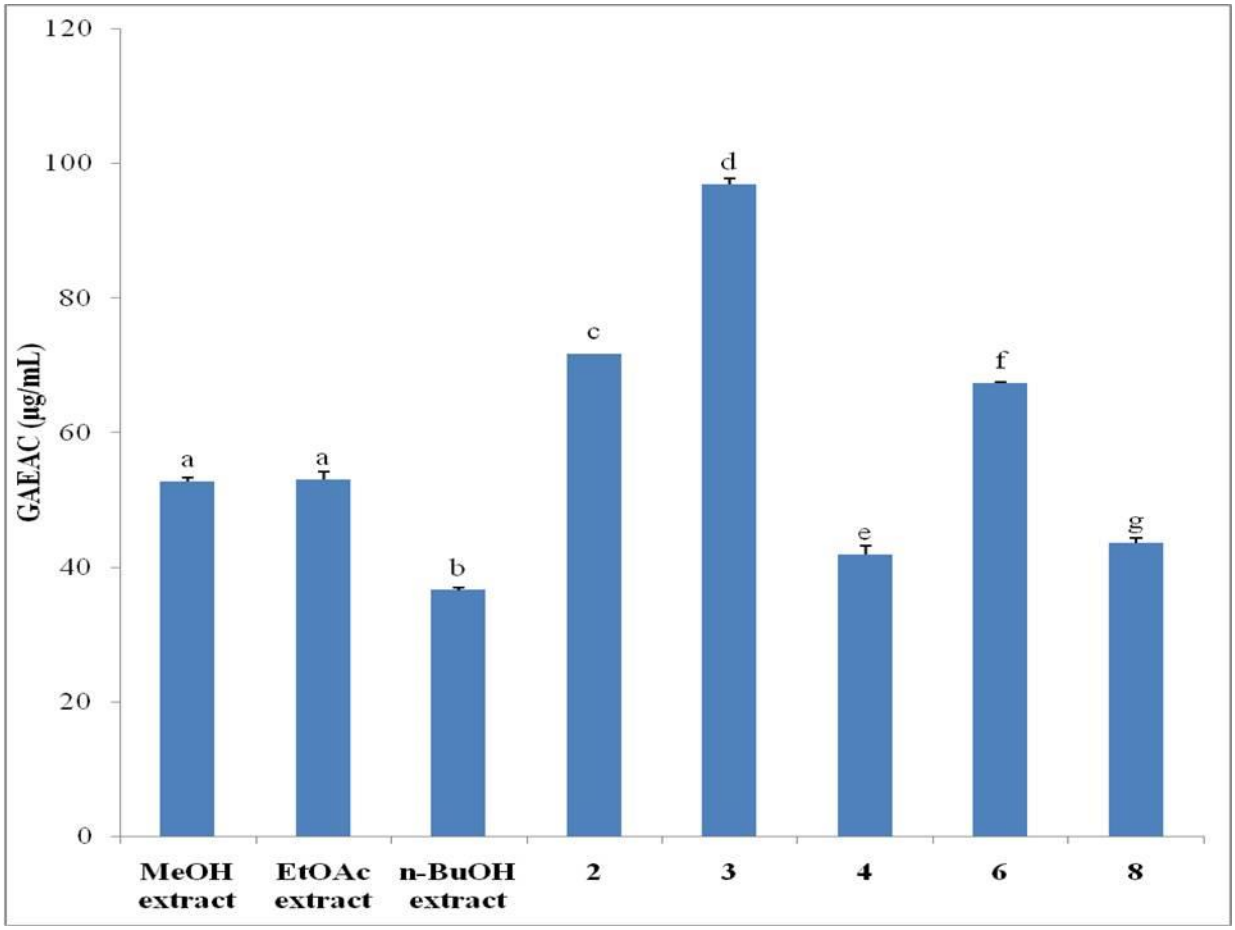


Figure 2. Gallic acid equivalent antioxidant capacity (GAEAC; µg/ml) of tested samples

Bars represent the mean ± SD of three independent experiments carried out in triplicate. Letters a-e indicate significant differences between samples according to one way ANOVA and Waller Duncan test; $p<0.05$. Compounds **1, 5, 7, 9** and **10** were not active (results not shown).

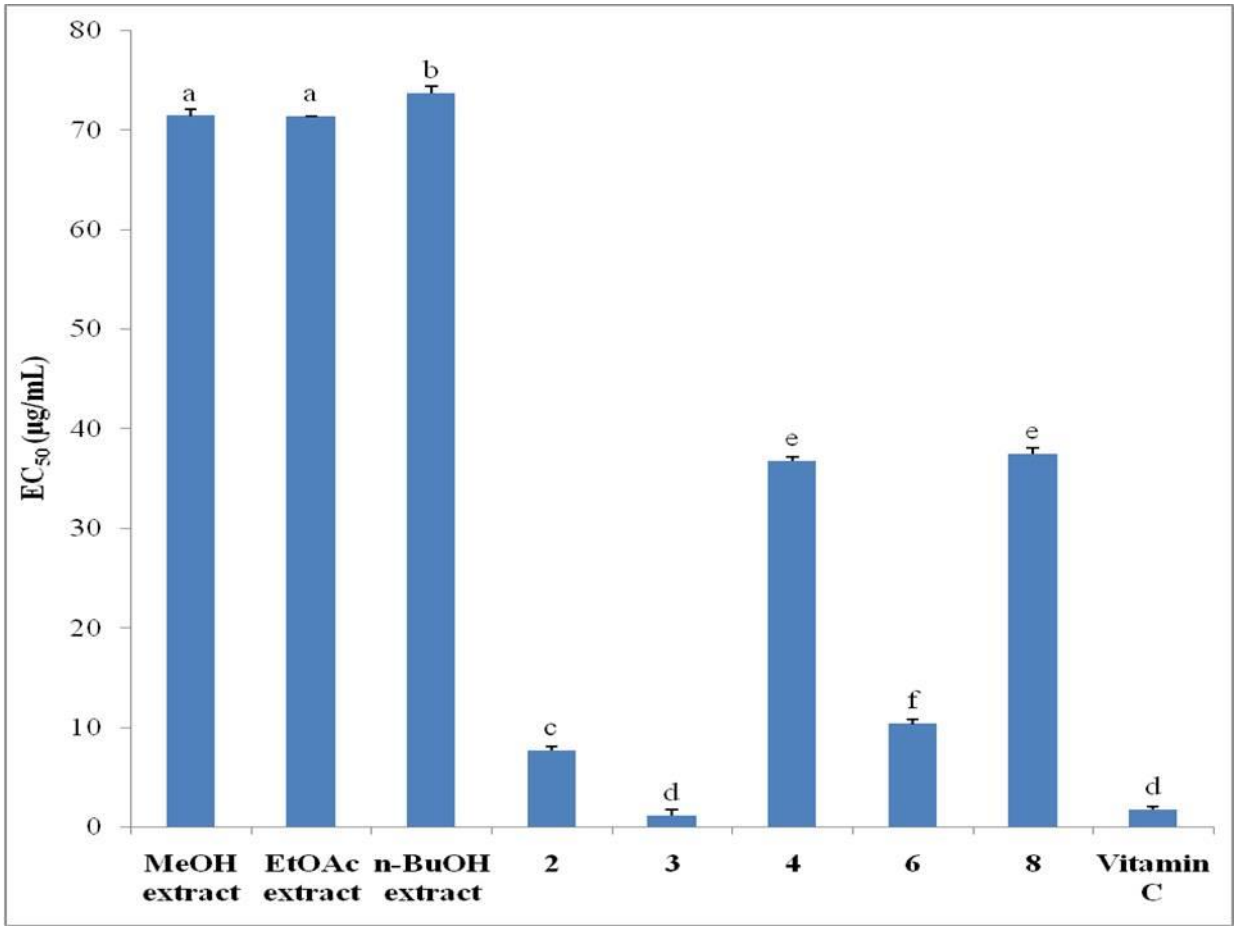


Figure 3. Equivalent concentrations of test samples scavenging 50% of DPPH radical (EC_{50}). Bars represent the mean ± SD of three independent experiments carried out in triplicate. Letters a-f indicate significant differences between samples according to one way ANOVA and Waller Duncan test; $p<0.05$. Compounds **1, 5, 7, 9** and **10** were not active (results not shown).