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# Saponins and Flavonoids from *Ludwigia leptocarpa* (Nutt) Hara (Onagraceae): Isolation, Characterization, Antibacterial and Antioxidant Activities

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## Abstract

*Diarrhea continues to be one of the most common causes of morbidity and mortality among infants and children in developing countries. The most common microorganisms responsible for diarrhoeal diseases are Vibrio cholerae, Escherichia coli and Shigella spp. 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 MeOH 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 six 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 trolox equivalent antioxidant capacity (TEAC) 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. Compounds 2, 3 and 6 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, Phenolic Acid, Triterpenoids, Flavonoids, Antibacterial, Antioxidant

## Introduction

Nowadays, treatments of many diseases with high mortality rates are still to be found. In some cases, the existing treatments are not adequate because patients with malaria, cholera or AIDS often die because of the high cost of treatment. Resistance to the most used medicament spread. The search for new molecules, more active and less expensive, with little or no side effects is an emergency for humans today.

Thus, plants have always been an essential source of medicines and therefore the majority of the world's population, especially in developing countries, is treated only with traditional herbal concoctions. From aspirin to Taxol, the modern pharmaceutical industry itself still relies heavily on the diversity of secondary metabolites to seek new molecules. This reserve is important since only a small portion (10%) of the 400,000 known plant species have been studied chemically and pharmacologically, and these species may contain several thousand different constituents [1].

Special attention should subsequently be paid to as many species as possible, which have not yet been the subject of phytochemical and pharmacological studies. Convinced that much remains to be done, the Laboratory of Applied and Environmental Chemistry (LACAPE) of the University of Dschang has undertaken to fully investigate Cameroonian medicinal plants like the *Ludwigia leptocarpa*.

*Ludwigia leptocarpa* (Onagraceae or Oenotheraceae family) is an herbaceous specie that is also readily found in North America and tropical Africa [2]. In the traditional medicine in Nigeria, an infusion of the plant is part of a mixture used to treat rheumatism. A leaf infusion has laxative, vermifugal and antidysenteric properties. Previous studies of this genus have revealed the presence of flavonoids, cerebrosides and triterpenoids [3,4]. A study recently reported that alcoholic extracts of *L. octovalvis*, *L. abyssinica*, *L. decurrens* and *L. Leptocarpa*, potentially have antioxidant, antibacterial and antifungal activities [2,5]. In the present paper, we report the isolation and characterization of the flavonoids from the MeOH extract of this plant, in order to evaluate their antibacterial and antioxidant activities.

## Methodology

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.

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 EtOAc and *n*-BuOH which were concentrated to dryness under reduced pressure to afford EtOAc (20 g) and *n*-BuOH (40 g) extracts, respectively.

Separation and isolation of compounds from the obtained extracts were performed using different chromatographic methods while the structures of the isolates were elucidated by interpretation of their spectroscopic data and by comparison of these data with those reported in the literature.

The extracts and six isolated compounds were evaluated for their antibacterial (against six bacterial strains: *Staphylococcus aureus* ATCC 25923, *Vibrio cholerae* SG24 (1), *Vibrio cholera* CO6, *Vibrio cholera* NB2, *Vibrio cholerae* PC2, *Shigellaflexneri* (SDINT) and antioxidant (using DPPH$^{\cdot}$ and ABTS$^{\cdot+}$ radicals) activities. MIC and MBC of extracts and compounds were assessed using the broth microdilution method [6].

The free radical scavenging activity of extracts as well as their isolated compounds was performed according to Brand-Williams et al. method (Brand-Williams 1995) [7]. The GEAC test was done as previously described by Rice-Evans and Miller in 1994 [8].

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 E (3.1 g) was purified over silica gel column chromatography eluted with the mixture hexane-EtOAc (6:4) to give compound 6 (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 $G_2$ (3.1 g) was purified over silica gel column chromatography eluted with the mixture EtOAc-MeOH (8.5:1.5) to give compounds 5 (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 1 (38 mg) and 2 (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 3 (66 mg) and 4 (40 mg).

## Results and discussion

### Chemical analysis

According to the antibacterial assays from MeOH, EtOAc and *n*-BuOH extracts, the EtOAc and *n*-BuOH extracts were submitted to further separation and purification. This led to the isolation of ten compounds. Structures (Figure 1) of these compounds have been assigned on the basis of spectroscopic data ($^1$H and $^{13}$C NMR, $^1$H-$^1$H COSY, HSQC, HMBC, ROESY and NOESY), mass spectrometry, and by comparison to their data with those of the literature. Hence, the isolated compounds were identified as:

28-*O*-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[α-L-arabinopyrano-syl-(1→3)]-4-O-(3'-hydroxybutanoyloxy-3-hydroxybutanoyloxy)-*β*-D-fucopyranosyl zanhic acid (1): 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) [4].

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 (2): 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) [4].

3-*O*-*β*-D-glucopyranosyl-28-*O*-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyrano-syl(1→2)-[α-L-arabinopyranosyl-(→3)]-4-O-(3'-hydroxybutanoyloxy-3-hydroxybutanoy-loxy)-*β*-D-fucopyranosyl zanhic acid (3): 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].

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'-hydroxybuta-noyloxy-3-hydroxybutanoyloxy)-β-D-fucopyranosyl zanhic acid (4): 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) [5].

(2R,3S,2''S)-3''',4',4''',5,5'',7,7''-heptahydroxy-3,8''-biflavanone (5): white amorphous powder from hexane-EtOAc; $C_{30}H_{22}O_{11}$; HRESIMS (positive-ion mode) m/z: 581.1057 $[M+Na]^+$ (calcd. for $C_{30}H_{22}O_{11}Na$: 581.1060) [9].

Luteolin-8-*C*-*glucoside* (6): yellow amorphous powder from EtOAc; $C_{21}H_{20}O_{11}$; HRESIMS (positive-ion mode) m/z: 471.0906 $[M+Na]^+$ (calcd. for $C_{21}H_{20}O_{11}Na$: 471.0903) [10].

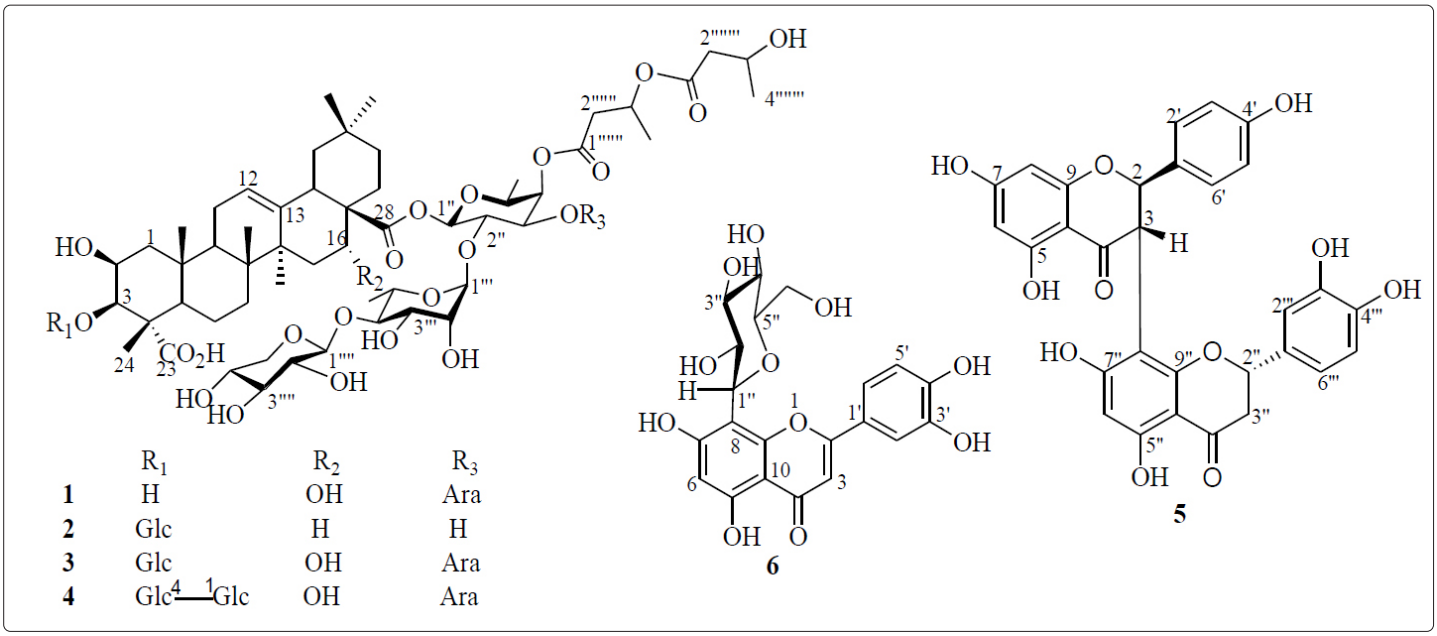


**Figure 1:** Structures of compounds isolated from the whole plant of *L. leptocarpa*

## Antibacterial activity

The susceptibility pattern and inhibition parameters of the tested organisms to the extracts and isolated compounds are indicated below (Table 1). The wells containing a concentration of 64-512 µg/ml of MeOH, EtOAc and *n*-BuOH extracts inhibited the visible growth of all the bacterial species. The most sensitive bacterial species were *S. aureus* and *S. flexneri*, while *V.cholerae* SG24 (1) and *V. cholerae* NB2 were the most resistant species to tested samples. MeOH extract showed the lowest antibacterial activity when compared with the EtOAc and *n*-BuOH extracts. This suggests that the fractionation of the MeOH extact enhanced its antibacterial activity. All the three plant extracts showed less antibacterial activity when compared with tetracycline. However, these extracts were active against *V. cholerae* NB2, *V. cholerae* 2 and *S. flexneri* which were not sensitive to ampicillin. The antimicrobial activity of plant extract was considered to be good if its MIC was less than 100.0 µg/ml, moderate if MIC was from 100.0 to 500.0 µg/ml and poor over 500.0 µg/ml [11]. Hence, the MeOH, EtOAc and *n*-BuOH extracts of *L. leptocarpa* exhibited good activity at a MIC value of 64 µg/ml against *S. aureus* whereas only the MeOH extract displayed poor activity against *V. cholerae* SG24 (1). The results of the *L. leptocarpa* extracts showed that this plant species is potential source of antibacterial agents. This *in vitro* study corroborated the previous antibacterial activities of alcoholic extracts from *L. octovalvis*, *L. abyssinica* and *L. decurrens* leaves against *Staphylococcus aureus* [2,5,12].

The lowest MIC and MBC values of 2 µg/mL were recorded on *S. aureus* with compound 6; highlighting its good antibacterial potential, as the activity on *S. aureus* was higher than that of ampicillin (MIC= 16 µg/mL and MBC = 16 µg/mL) and tetracycline (MIC = 16 µg/mL and MBC = 128 µg/mL) used as reference antibacterial drugs. However, the highest MIC value of 512 µg/mL was recorded on *V. cholerae* SG24 (1) with MeOH extract, and the highest MBC value of 512 µg/mL was obtained on *V. cholerae* SG24 (1), *V. cholerae* CO6 and *V. cholerae* 2 with the MeOH extract. A lower MBC/MIC (≤4) value signifies that a minimum amount of plant extract/ isolated compound is used to kill the bacterial species, whereas, a higher values signifies the use of comparatively higher concentration of the compounds is needed for the control of the microorganism [13].

The antibacterial activities of isolated compounds from *L. leptocarpa* are in the order as compound 5 > compound 6 > compounds 2, 3 > compound 4 > compound 1. Compounds 5, 6, 2, 3 and 4 were active against all the tested pathogens. Antimicrobial cutoff points have been defined by several authors to enable the understanding of the potential of pure compounds as follows: significant activity (MIC < 10 µg/mL), moderate activity (10 < MIC ≤ 100 µg/mL), and low activity (MIC > 100 µg/mL) [14,15]. Based on this, the antibacterial activity of compound 6 on *V. cholerae* CO6, *V. cholerae* NB2, *V. cholerae* 2, *S. flexneri* and *S. aureus* as well as that of compound 5 on *S. flexneri* SDINT and *S. aureus* ATCC 25923 can be considered significant. Ampicillin was not active against *V. cholerae* NB2, *V. cholerae* PC2, *S. flexneri* at concentrations up to 512 µg/mL.

**Table 1: Antibacterial activity (MIC and MBC in µg/ml) of extracts, isolated compounds and reference antibacterial drugs**

| Extracts/ compounds | Inhibition parameters | *Vibrio cholerae* SG24 (1) | *Vibrio cholerae* CO6 | *Vibrio cholerae* NB2 | *Vibrio cholerae* 2 | *Shigella flexneri* SDINT | *Staphylococcus aureus* ATCC 25923 |
|---|---|---|---|---|---|---|---|
| MeOH | MIC | 512 | 256 | 256 | 256 | 128 | 64 |
| extract | MBC | 512 | 512 | 256 | 512 | 128 | 128 |
| | MBC/MIC | 1 | 2 | 1 | 2 | 1 | 2 |
| EtOAc | MIC | 128 | 256 | 128 | 128 | 128 | 64 |
| extract | MBC | 256 | 256 | >512 | 256 | 128 | 128 |
| | MBC/MIC | 2 | 1 | / | 2 | 1 | 2 |
| *n*-BuOH | MIC | 256 | 256 | 128 | 256 | 128 | 64 |
| extract | MBC | 256 | >512 | 256 | 256 | 128 | 128 |
| | MBC/MIC | 1 | / | 2 | 1 | 1 | 2 |
| 1 | MIC | >256 | 256 | 256 | 256 | 128 | 128 |
| | MBC | / | >256 | >256 | >256 | >256 | 128 |
| | MBC/MIC | / | / | / | / | / | 1 |
| 2 | MIC | 128 | 256 | 128 | 128 | 128 | 64 |
| | MBC | >512 | 256 | 256 | 256 | 128 | 64 |
| | MBC/MIC | / | 1 | 2 | 2 | 1 | 1 |
| 3 | MIC | 256 | 128 | 128 | 128 | 64 | 64 |
| | MBC | >256 | 256 | 128 | 256 | 128 | 64 |
| | MBC/MIC | / | 2 | 1 | 2 | 2 | 1 |
| 4 | MIC | 256 | 256 | 256 | 256 | 128 | 128 |
| | MBC | >256 | >256 | >256 | >256 | >256 | 128 |
| | MBC/MIC | / | / | / | / | / | 1 |
| 5 | MIC | 16 | 8 | 8 | 8 | 4 | 2 |
| | MBC | 16 | 8 | 8 | 8 | 4 | 2 |
| | MBC/MIC | 1 | 1 | 1 | 1 | 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 |
| Ampicillin | MIC | 16 | 16 | >512 | >512 | >512 | 16 |
| | MBC | 16 | 16 | >512 | >512 | >512 | 16 |
| | MBC/MIC | 1 | 1 | / | / | / | 1 |
| Tetracycline | MIC | 0.5 | 2 | 0.5 | 0.5 | 16 | 16 |
| | MBC | 4 | 16 | 4 | 4 | 128 | 128 |
| | MBC/MIC | 8 | 8 | 8 | 8 | 8 | 8 |

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

However, most of the tested samples displayed antibacterial activities against these microbial strains; suggesting that their administration may represent an alternative treatment against the *V. cholerae*, the causative agent of dreadful disease cholera and *S. flexneri*, the causative agent of shigellosis. Taking into account the medical importance of the tested bacteria, this result can be considered as promising in the perspective of new antibacterial drugs development. The antibacterial activities of oleanolic acid, ellagic acid and 2*β*-hydroxyoleanolic acid corroborate those of the early reports [16,17]. 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 [13,18]. The strains of *V. cholerae* and *S. flexneri* included in the present study were MDR clinical isolates and these were resistant to commonly used drugs such as ampicillin, streptomycin, nalidixic acid, furazolidone, co-trimoxazole, etc.

The mechanism of action of saponins (1 - 4) is not fully understood, but is speculated to involve membrane disruption by the lipophilic compounds [19]. The mechanism of the flavonoids (5, 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 [20].

## 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· and ABTS·+ 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 [13,21]. The compounds, which showed the strongest DPPH· and ABTS·+ radical scavenging activities, are compounds 5 ($EC_{50}$ = 1.09 µg/mL; TEAC= 96.88 µg/mL) and 6 ($EC_{50}$ = 10.34 µg/mL; TEAC= 67.35 µg/mL), while the compound 2 shows moderate antioxidant properties. Compounds 1, 3 and 4 were found not active in both the two model systems. Compound 6 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 5 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.

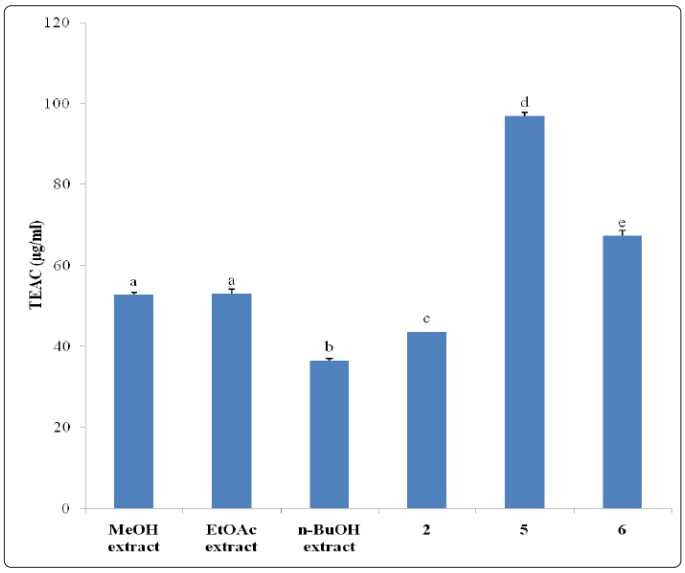


**Figure 2:** Gallic acid equivalent antioxidant capacity (TEAC; µg/ml) of tested samples

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, 3 and 4 were not active (results not shown).

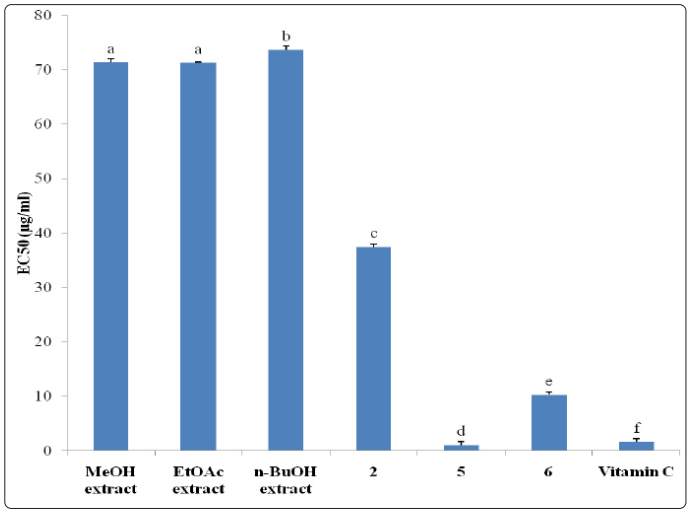


**Figure 3:** Equivalent concentrations of test samples scavenging 50% of DPPH radical ($EC_{50}$).

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, 3 and 4 were not active (results not shown).

## Conclusion

The results show that the MeOH and EtOAc extracts from *L. leptocarpa* as well as compounds 5 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 [22-44].


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## References


1. OsmancK, Evangelopoulos, D, Basavannacharya C, Gupta A, McHugh TD, et al. (2012) An antibacterial from Hypericum acmosepalum inhibits ATP-dependent MurE ligase from Mycobacterium tuberculosis. Int. J. Antimicrob. Agents. 39: 124-129.
2. Oyedeji O, Oziegbe M, Taiwo FO (2011) Antibacterial, antifungal and phytochemical analysis of crude extracts from the leaves of Ludwigia abyssinica A. Rich. and Ludwigia decurrens Walter. J. Med. Plant. Res 5:1192-1199.
3. Mabou FD, Foning TPL, Ngnokam D, Harakat D, Voutquenne-Nazabadioko L(2014) Leptocarposide: a new triterpenoid glycoside from Ludwigia leptocarpa (Onagraceae). Magn. Reson. Chem 52: 32-36.
4. Mabou FD, Ngnokam D, Harakat D, Voutquenne-Nazabadioko L (2015) New oleanane-type saponins: Leptocarposide B-D, from Ludwigia leptocarpa (Onagraceae). Phytochem. Lett 14: 159-164.
5. Yakob HK, Sulaiman SF, Uyub AM (2012) Antioxidant and Antibacterial Activity of Ludwigia octovalvis on Escherichia coli O157:H7 and some pathogenic bacteria. World Applied Sciences Journal 16: 22-29.
6. NCCLS (1999) Methods for determining bactericidal activity of antimicrobial agents. Approved guideline, M26-A. National

Committee for Clinical Laboratory Standards, Wayne.
7. Brand-Williams W, Cuvelier M, Berset C, (1995) Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft Und-Technologie-Food Science and Technology 28: 25-30.
8. Rice-Evans C, Miller NJ (1994) Total antioxidant status in plasma and body fluids. Methods Enzymol. 234: 279-293.
9. Messi BB, Ndjoko-Ioset K, Hertlein-Amslinger B, Meli A, Nkengfack, AE, et al. (2012) Preussianone, a new flavanone-chromone biflavonoid from Garcinia preussii Engl. Molecules 17: 6114-6125.
10. Koeppen BH (1965) C-Glycosylflavonoids. The chemistry of orientin and iso-orientin. Biochem. J 97: 444-448.
11. Dabur R, Gupta A, Mandal TK, Singh DD, Bajpai V (2007) Antimicrobial activity of some indian medicinal plants. Afr. J. Trad. CAM 4: 313-318.
12. Oyedeji O, Taiwo FO, Ayinde FO, Ajayi OS, Oziegbe, et al. (2014) In vitro Antimicrobial and antioxidant analysis of gallic acid from the leaves of Ludwigia abyssinica A. Rich. European J. Med. Plants 4:1098-1112.
13. Djouossi MG, Tamokou JDD, Ngnokam D, Kuiate JR, Tapondjou AL, et al. (2015) Antimicrobial and antioxidant flavonoids from the leaves of Oncoba spinosa Forssk. (Salicaceae). BMC Compl Altern Med 15: 134-152.
14. Kuete V (2010) Potential of Cameroonian plants and derived products against microbial infections: a review. Planta Medica 76: 1479-1491.
15. Kuete V, Efferth T (2010) Cameroonian medicinal plants: pharmacology and derived natural products. Front Pharmacol 1: 123.
16. Wansi JD, Chiozem DD, Tcho AT, Toze FA, Devkota, et al. (2010) Antimicrobial and antioxidant effects of phenolic constituents from Klainedoxa gabonensis. Pharm. Biol 48: 1124-1133.
17. Simpol L, Dayrit F, Abe F, Yamauchi T (1993) Isolation of maslinic acid from the leaves of Bixa orellana. Philippine J. Sci 118: 147-166.
18. Garcia A, Bocanegra-Garcia V, Palma-Nicolas JP, Rivera G (2012) Recent advances in antitubercular natural products. European J. Med. Chem 49: 1-23.
19. Termentzi A, Fokialakis N, Skaltsounis AL (2011) Natural resins and bioactive natural products thereof as potential antimicrobial agents. Curr. Pharm. Des 17: 1267-1290.
20. Cowan MM (1999) Plant product as antimicrobial agents. Clinical Microbiology Reviews 12: 564-582.
21. Shikanga OT, Mutonga D, Abade M, Amwayi S, Ope M, et al. (2009) High mortality in a cholera outbreak in western Kenya after post-election violence in 2008. Am. J. Trop. Med. Hyg 81: 1085-1090.
22. Akinmoladun AC, Obuotor EM, Farombi EO (2010) Evaluation of antioxidant and free radical scavenging capacities of some Nigerian indigenous medicinal plants. J Med Food 13: 444-451.
23. Averett JE, Zardini EM, Hoch PC (1990) Flavonoid systematic of ten sections of Ludwigia (Onagraceae). Biochem. Syst. Ecol 18: 529-532.
24. Ayinampudi SR, Ramchander M, Thirupathaiah A (2013) A New Triterpene from Ludwigia hyssopifolia (G. Don) Exell. International Journal of Chem Tech Research 5: 342-346.
25. Bhattacharya SK, Bhattacharya MK, Nair GB, Dutta D, Deb A, et al. (1993) Clinical profile of acute diarrhoea cases infected with the new epidemic strain of Vibrio cholerae O139: designation of the disease as cholera. J. Infect 27: 11-15.
26. Borris RP (1996) Natural products research: perspectives from a major pharmaceutical company. J. Ethnopharmacol 51: 29-38.
27. Cai Y, Luo Q, Sun M, Corke H (2004) Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci 74: 2157-2184.
28. Cariri FAMO, Costa APP, Melo CC, Theophilo GND, Hofer E, et al. (2010) Characterization of potentially virulent non-O1/non-O139 Vibrio cholerae strains isolated from human patients. Clin Micro Infec 16: 62-67.
29. Dragland S, Senoo H, Wake K, Holte K, Blomhoff R (2003) Several culinary and medicinal herbs are important sources of dietary antioxidants. J. Nutr 133: 1286-1290.
30. Fennell CW, Lindsey KL, McGaw LJ, Sprag SG, Staffort GI, et al. (2004) Assessing African medicinal plants for efficacy and safety: pharmacological screening and toxicity. J. Ethnopharmacol 94 : 205-217.
31. Kaper JB, Morris JG, Levine MM (1995) Cholera. Clin. Microbiol Rev 8: 48-86.
32. Kundu AP, Mahato SB (1993) Triterpenoids and their glycosides from Terminalia chebula. Phytochemistry 32: 999-1002.
33. Marzouk MS, Moharram FA, El-Dib RA, El-Shenawy SM, Tawfike AF (2009) Polyphenolic Profile and Bioactivity Study of Oenothera speciosa Nutt. Aerial Parts. Molecules 14: 1456-1467.
34. Mot AC, Pârvu M, Damian G, Irimie FD, Darula Z, et al. (2012) A “yellow” laccase with “blue” spectroscopic features, from Sclerotinia sclerotiorum. Process Biochem 47: 968-975.
35. Özen T, Çöllü Z, Korkmaz H (2010) Antioxidant Properties of Urtica pilulifera root, seed, flower, and leaf extract. J. Med. Food 13:1224-1231.
36. Oziegbe M, Faluyi JL (2012) Reproductive biology of Ludwigia leptocarpa and L. adscendans subsp. Diffusa in Ile Ife, Nigeria. Turk J. Botanic 36: 162-173.
37. Zwane PA, Kremer M (2007) What works in fighting diarrheal diseases in developing countries? A critical review. The World Bank Res. Observer 22: 1-24.
38. Rice LB (2006) Unmet medical needs in antibacterial therapy. Biochem. Pharmacol 71: 991-995.
39. Sasaki S, Suzuki H, Fujino Y, Kimura Y, Cheelo M (2009) Impact of drainage networks on cholera outbreaks in Lusaka, Zambia. Am. J. Public. Health 99: 1982-1987.
40. Shikanga OT, Mutonga D, Abade M, Amwayi S, Ope M, et al. (2009) High mortality in a cholera outbreak in western Kenya after post-election violence in 2008. Am. J. Trop. Med. Hyg 81: 1085-1090.

41. Tamokou JDD, Kuiate JR, Tene M, Nwemeguela KTJ, Tane P (2011) The antimicrobial activities of extract and compounds isolated from Brillantaisia lamium. Iranian J Med Sci 36: 24-31.
42. Taneja N, Kaur J, Sharma K, Singh M, Kalra JK, et al. (2003) A recent outbreak of cholera due to Vibrio cholerae O1 Ogawa in and around Chandigarh, north India. Indian J. Med. Res 117: 243-246.
43. World Health Organisation (WHO) (2010) Weekly Epidemiological Record. Cholera 86: 325-340.
44. World Health Organization (2009) Cholera: global surveillance summary, 2008. Wkly Epidemol. Rec 84: 307-331.

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