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***In vitro* Antimicrobial and Antioxidant Analysis of Gallic Acid from the Leaves of *Ludwigia abyssinica* A. Rich**

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OO, FOT and OSA designed the study. Author OO managed literature searches and wrote the first draft of manuscript. Authors FOT, OSA, MO and OO carried out the research in the laboratory. Author FOA managed the analyses of the study. Authors MTK and AHA performed the statistical analysis. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: To evaluate the antimicrobial and antioxidant potentials of a biologically active compound isolated from the leaves of *Ludwigia abyssinica* A. Rich.

Study Design: Antimicrobial and antioxidant analysis of compound from the *n*-butanol fraction of plant leaf.

Place and Duration of Study: Chemistry and Microbiology Laboratories, Faculty of Science, Obafemi Awolowo University, Ile-Ife between November 2011 and September 2012.

Methodology: Chromatographic and nuclear magnetic resonance techniques were used to isolate and characterize the biologically active compound, respectively. Disc diffusion, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) measurements were used for antimicrobial test. 1, 1-diphenyl-2-picrylhydrazyl hydrate (DPPH) radical scavenging assay was used for antioxidant test.

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Results: The phenolic compound, 3,4,5-trihydroxybenzoic acid, also known as gallic acid was isolated from the *n*-butanol fraction of *L. abyssinica* leaf. It showed broad spectrum antimicrobial activities with zones of inhibition in the range 26.67±1.53 to 32.00±1.00 mm for the test bacteria and 6.67±1.15 to 30.33±0.58 mm for the test fungal strains. MIC and MBC values for gallic acid ranged from 12.5 to 200 µg ml⁻¹ and 50 to 400 µg ml⁻¹, respectively, comparing favourably with the standard reference drug used. DPPH radical scavenging activity for the compound was IC₅₀ = 9.38 µg ml⁻¹ compared with IC₅₀ = 27.08 µg ml⁻¹ obtained for the standard ascorbic acid.

Conclusion: The results show that gallic acid from *Ludwigia abyssinica* leaves possesses *In vitro* antimicrobial and antioxidant properties and thus the plant has potentials as a source of natural health-giving products, given further investigations.

Keywords: Antimicrobial and antioxidant activities; *Ludwigia abyssinica*; gallic acid; phenolic compound.

1. INTRODUCTION

Extracts from diverse species of plants continue to be used as herbal remedies by various human traditional settings [1,2]. The antimicrobial activities of such plant extracts form the basis of their use in alternative medicines and natural therapies [3,4]. There is a marked increase in the development of resistance to the existing antimicrobial agents by microbes [5,6]. Biologically active chemical compounds of plants have attracted attention as natural products that could be used in the development of new antimicrobial agents effective against antibiotic-resistant and pathogenic bacteria and fungi [1].

Phytochemical compounds also provide basis for the development of medicaments against human ailments such as cancer, ulcerative colitis and infectious diseases [2,7,8]. Oxidative stress is involved in the development of chronic and degenerative diseases such as aging, cancer, arteriosclerosis, malaria, cardiovascular, inflammatory, cerebrovascular and immune deficiency diseases [9,10,11,12]. Medicinal plants are increasingly investigated as natural sources of antioxidants for reducing oxidative damages associated with these diseases. Researchers have become more interested in their polyphenol constituents due to their potent antioxidant properties and their credible effects in the prevention of various oxidative stress-associated diseases [13]. 3, 4, 5-Trihydroxybenzoic acid (gallic acid) is a naturally occurring plant phenolic compound found both in its free state and as part of a tannin molecule [14,15]. Either in its free or binded form, the compound possesses anticancer, anti-inflammatory, neuroprotective and analgesic effects [15,16,17,18].

Ludwigia abyssinica A. Rich. (Onagraceae) is native to South America but is now well distributed in Africa [19,20]. It is an aquatic herb growing in ditches, shallow marshy areas, river banks and slow moving streams. It is useful as an ornamental plant because of its showy flowers which are yellow in color, and sold in many parts of Europe [21]. The seeds are very rich in oil [22].

Extracts of the leaves and roots of the plant are used in traditional medicine to treat ailments such as cough, skin diseases, sores, diarrhea, dysentery, cholera, flatulence and constipation [20,23]. Aqueous decoction of the leaves is taken orally for its analgesic effect in treatment of generalized pain in some parts of Africa [23]. Leaf decoctions of the plant are also used in the treatment of fungal infections by the Haya people of Northwestern Tanzania [24]. The leaf sap is taken orally to stave off threatened abortion [20]. In East Africa, a root

decoction of the plant is used to treat liver diseases and intestinal worm infestations in children while the leaves are eaten as cooked vegetable for its nutritional values in the Democratic Republic of Congo, Malawi, parts of Nigeria and Tanzania [20,24,25].

In a previous study, crude extracts of *L. abyssinica* leaves were found to exhibit considerable antimicrobial effects when compared with standard antibiotic and antifungal agents [26]. As far as we know, there are no previous studies regarding the biological effects of chemical constituents of this plant. The present work therefore investigates and reports the antioxidant and antimicrobial properties of a phenolic compound isolated from the *n*-butanol fraction of the leaves.

2. MATERIALS AND METHODS

2.1 Plant Material

L. abyssinica leaves were collected from several ditches in the Obafemi Awolowo University, Ile-Ife, Nigeria campus in November, 2011 and authenticated taxonomically by Dr. Matthew Oziegbe at the Ife herbarium in the University. A voucher specimen number U.H.I 16192 was preserved at the Ife herbarium for future reference.

2.2 Extraction

The collected plant was air dried for three weeks and then blended into powder. Extractions were performed by maceration in which the powdered *L. abyssinica* leaves (900 g) were soaked in 50% aqueous-methanol (5 L) at room temperature for 72 h [27]. The extracts were then filtered using Whatman filter paper no. 2 and concentrated *in vacuo* at 40°C on a rotary evaporator (Heldolph, Germany) to about one-third of its original volume. Concentrated crude extract of the plant was in turn dissolved in distilled water and partitioned with *n*-hexane (3×1 L), dichloromethane (3×1 L), ethyl acetate (3×1 L) and *n*-butanol (3×0.7 L). The partitioned fractions were concentrated to dryness *in vacuo* to afford four different fractions: hexane, dichloromethane, ethyl acetate, and *n*-butanol fractions with compounds of appropriate polarity.

2.3 Bacterial and Fungal Species

The test bacteria species were *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 19582), *Escherichia coli* (ATCC 8739), *Enterococcus faecalis* (ATCC 29212), *Bacillus subtilis* (ATCC 6633), *B. stearothermophilus*, *Klebsiella pneumonia*, *Clostridium sporogenes*, *Salmonella typhi* and *Serratia marcescens* (ATCC 9986). The test fungal species were *Aspergillus niger* (ATCC 6275), *A. flavus*, *Penicillium camemberti*, *Fusarium oxysporium*, *Trichophyton mentagrophytes* and *Candida albicans* (ATCC 10231). They were obtained from the culture collection of the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. The bacterial species included reference strains (6), environmental strains (2) and clinical isolates (2). The fungal species included reference strains (2) and soil environmental strains (4).

2.4 Isolation of Compound

Butanol fraction (10.29 g) was fractionated by column chromatography on silica gel (60-120 mesh) on glass column (length 60 cm; diameter 4.5 cm) using *n*-hexane as the eluant. This

was followed by an increasing gradient of ethyl acetate up to 100% and in turn by an increasing gradient of methanol up to 100%. One hundred and nine test tube fractions of 15 ml each were collected. Analysis of these test tube fractions on TLC plate using ethyl acetate/methanol (4:1) gave seven fractions (1A-1G). Fraction 1A (600 mg) was purified on sephadex LH-20 using ethyl acetate/methanol (9:1) followed by an increasing gradient of methanol up to 100%. Two fractions 2A (270 mg) and 2B were obtained on analysis on TLC plate using ethyl acetate/methanol (4:1) as the solvent system. Purification of 2B (300 mg) on sephadex LH-20 using ethyl acetate/methanol (9.5:0.5) followed by an increasing gradient of methanol up to 100% gave the isolated compound (139 mg) with melting point 230-231°C.

2.5 Structural Elucidation of Compound

Partial structural elucidation of the isolated compound from *n*-butanol fraction was carried out using ^1H and ^{13}C Nuclear Magnetic Resonance spectroscopic analysis.

2.6 Antimicrobial Assays

2.6.1 Preparation of inocula

The bacterial strains grown on nutrient agar at 37°C for 18 h were suspended in sterile normal saline solution (0.85% w/v sodium chloride) and adjusted to a turbidity of 0.5 McFarland standard (10^8 CFU/ml). The suspensions were diluted 100 times with Mueller Hinton broth to give 10^6 CFU/ml [28]. Suspensions of fungal spores from fresh cultures were prepared in sterilized 0.85% w/v sodium chloride solutions, which were compared with McFarland solution. These were then diluted to approximately 10^6 CFU/ml.

2.6.2 Disc diffusion assay

Antimicrobial activities were determined by the agar diffusion method [29], with modifications. Mueller Hinton agar and SDA were inoculated with microbial cell suspensions (200 μl in 20 ml medium) and poured into sterile petri dishes. Stock solutions of isolated compound were dissolved in 10% DMSO solution to give a final concentration of 1000 $\mu\text{g/ml}$. The solutions were then sterilized by filtration through 0.45 μm millipore filters. Sterile paper discs (6 mm) were impregnated with 10 μl of the sterile solutions of the isolated compound and placed on the agar surface. Standard discs (6 mm) containing the broad spectrum antibiotic, streptomycin (10 $\mu\text{g/disc}$) (Oxoid, UK), and antifungal agent amphotericin B (10 $\mu\text{g/disc}$) (Abtek Biologicals Ltd, UK), were used as positive controls. Discs impregnated with 10% DMSO were used as negative control. The plates were incubated overnight at 37°C for 18-24 h. The plates of the fungal strains were incubated at 31°C for 72 h. The experiment was tested in triplicates and zones of growth inhibition were recorded in millimetres.

2.6.3 Micro-well dilution assay

2.6.3.1 Minimum inhibitory concentrations (MIC)

Minimum inhibitory concentrations of the isolated compound from *L. abyssinica* were determined based on the broth microdilution assay [30]. The dried isolated compound was dissolved in 10% DMSO in water to give a concentration of 400 $\mu\text{g/ml}$. The broad spectrum antibiotic, streptomycin, which was used as positive control, was similarly treated. Serial two

fold dilutions were then made in a concentration range 12.5 to 400.0 µg/ml. The 96-well microtiter plates were prepared by dispensing into each well 100 µl of Mueller Hinton broth. Inoculum (10 µl) of each bacteria and 50 µl (0.2 mg/ml) of p-iodonitrotetrazolium chloride (INT) were then added into each well. The plates were covered with parafilm, shaken to mix the contents and then incubated at 37°C for 24 h. The MIC was defined as the lowest concentration at which no visible growth was observed. The colourless tetrazolium salt acts as an electron acceptor and is reduced to a red-colored formazan product by biologically active organisms [31]. Where bacterial growth was inhibited, the solution in the well remained clear after incubation with INT. Each experiment was done in triplicates.

2.6.3.2 Minimum bactericidal concentration (MBC)

Minimum bactericidal concentrations (MBC) of the isolated compound were determined by removing 100 µl of bacterial suspension from subculture demonstrating no visible growth and inoculating this on Mueller Hinton agar plates. Plates were incubated at 37°C for 24 h with experiment being carried out in triplicates.

2.7 Antioxidant Activity

The free radical scavenging activity of the isolated compound was evaluated by using 1,1-diphenyl-2-picrylhydrazyl hydrate (DPPH) [32]. Different concentrations (5 to 60 µg ml⁻¹) of the isolated compound were pipetted to the test tubes and volume adjusted to 3 mL with ethanol. 0.1 mM Alcoholic DPPH solution (1.0 mL) was added to the sample. The sample was vortexed, and incubated in dark at room temperature for 30 min. The absorbance was measured at 517 nm against blank samples. Decreased absorbance of the sample indicates DPPH free radical scavenging capability [33]. Ascorbic acid (vitamin c) was used as reference positive control. The DPPH free radical scavenging activity was calculated using the expression:

$$\% \text{ DPPH free radical scavenging activity} = [1 - A_{517 \text{ nm of sample}}/A_{517 \text{ nm of control}}] \times 100\%$$

3. RESULTS

3.1 Structural Elucidation of the Isolated Compound

The ¹H NMR shows signals at δ 7.0 ppm, which indicates the presence of aromatic protons. The ¹³C NMR showed a signal at δ 169 ppm which represents the carboxylic acid carbon. Carbons 2 and 6 are in the same chemical environment hence, they have the same chemical shift which is the signal seen at δ 108 ppm. Carbons 3 and 5 are also in the same chemical environment and they account for the δ 144 ppm signal. Carbon 4 is however responsible for the signal at δ 138 ppm. Therefore, the isolated compound is 3, 4, 5-trihydroxybenzoic acid (gallic acid) (Fig. 1). It showed a single, clean spot on its TLC profile with a retention factor (R_f) of 0.5 in ethyl acetate/methanol (4:1). The compound is solid crystalline, off-white in color and has a melting point of 230-231°C. A little quantity of it, dissolved in methanol, gave an intense blue-black coloration on addition of freshly prepared ferric chloride solution hence confirming it to be a phenolic compound.

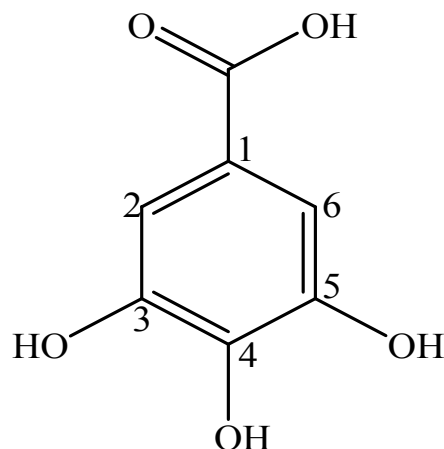


Fig. 1. Chemical structure of gallic acid isolated from *Ludwigia abyssinica* n-butanol fraction

3.2 Antimicrobial Activity

The results of the study of the antibacterial activity of gallic acid using the disc diffusion assay are presented in Table 1. The zones of inhibition ranged from 28.00±1.00 to 32.00±1.00 mm with the highest inhibitory effect being against *S. aureus* and the weakest activity demonstrated against *S. typhi*. The isolated compound was also more effective in the inhibition of all the test fungal species compared with the broad spectrum antifungal agent - amphotericin B. It produced zones of inhibition range 17.67±1.53 to 30.33±0.58 mm. It exhibited the greatest antifungal activity against *A. niger* and the weakest activity against *C. albicans* (Table 2). In view of the results obtained in the disc diffusion assay, the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of gallic acid were determined. The MIC values ranged between 12.5 and 100 µg ml⁻¹ while the MBC ranged between 50 and 200 µg ml⁻¹. The compound was most inhibitory against *E. faecalis*, *S. aureus* and *B. subtilis* but least inhibitory against *K. pneumonia*, *S. typhi* and *P. aeruginosa* (Table 3). The results obtained in the disc diffusion assay were found to correlate with that obtained in the MIC assay.

Table 1. Antibacterial activities of gallic acid isolated from *L. abyssinica* leaf

Test bacteria	Mean zones of inhibition in mm*	
	Gallic acid	Streptomycin
<i>S. aureus</i>	30.33±0.58	30.33±1.52
<i>E. faecalis</i>	29.67±0.58	32.00±0.00
<i>B. subtilis</i>	31.00±0.00	30.67±0.58
<i>E. coli</i>	30.33±0.58	23.67±0.58
<i>K. pneumonia</i>	30.00±1.00	29.00±1.00
<i>B. stearothermophilus</i>	30.33±0.58	30.67±1.53
<i>Cl. sporogenes</i>	30.67±0.58	31.00±1.73
<i>S. marcescens</i>	30.33±0.58	31.00±0.58
<i>S. typhi</i>	28.00±1.00	29.67±0.58
<i>P. aeruginosa</i>	28.66±0.58	26.67±1.15

mm* - Mean of three replicates ± standard deviation; Streptomycin (10 µg/disc)

Table 2. Antifungal activities of gallic acid isolated from *L. abyssinica* leaf

Test fungi	Mean zones of inhibition in mm*	
	Gallic acid	Amphotericin B
<i>A. niger</i>	30.33±0.58	22.67±1.15
<i>A. flavus</i>	29.67±0.58	24.30±1.15
<i>P. camemberti</i>	28.33±1.53	20.67±1.53
<i>F. oxysporium</i>	30.00±0.00	23.00±1.73
<i>T. mentagrophytes</i>	28.33±2.08	20.67±1.15
<i>C. albicans</i>	17.67±1.53	14.67±0.58

mm* - Mean of three replicates ± standard deviation. Amphotericin B (10 µg/disc)

Table 3. Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of gallic acid and reference antibiotic (streptomycin)

Test bacteria	Average MIC / MBC			
	Gallic acid		Streptomycin	
	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)
<i>S. aureus</i>	12.5±0.00	50.0±0.00	50.0±0.00	100.0±0.00
<i>E. faecalis</i>	12.5±0.00	50.0±0.00	50.0±0.00	50.0±0.00
<i>B. subtilis</i>	12.5±0.00	50.0±0.00	50.0±0.00	200.0±0.00
<i>E. coli</i>	50.0±0.00	200.0±0.00	50.0±0.00	200.0±0.00
<i>K. pneumonia</i>	50.0±0.00	200.0±0.00	50.0±0.00	100.0±0.00
<i>B. stearothermophilus</i>	25.0±0.00	100.0±0.00	50.0±0.00	100.0±0.00
<i>Cl. Sporogenes</i>	25.0±0.00	50.0±0.00	50.0±0.00	50.0±0.00
<i>Serratia marcescens</i>	25.0±0.00	100.0±0.00	25.0±0.00	100.0±0.00
<i>S. typhi</i>	100.0±0.00	100.0±0.00	50.0±0.00	200.0±0.00
<i>P. aeruginosa</i>	100.0±0.00	200.0±0.00	100.0±0.00	200.0±0.00

The results are means of three replicates ± standard deviation

3.3 Antioxidant Activity

Table 4 illustrates the percentage inhibition of DPPH radical by gallic acid and standard ascorbic acid. At a concentration of 60 µg ml⁻¹, the scavenging activity of gallic acid reached 95.63±0.21% compared with 80.51±0.53% produced by ascorbic acid. The IC₅₀ value was found to be 9.38 and 27.08 µg ml⁻¹ for gallic acid and ascorbic acid, respectively (Figs. 2 and 3).

Table 4. DPPH free radical scavenging activity of gallic acid isolated from *L. abyssinica* leaf

Concentration (µg/ml)	DPPH percentage inhibition (%)	
	Gallic acid	Ascorbic acid
5.0	31.13±0.63	2.52±0.05
10.0	52.38±0.61	6.82±0.23
15.0	74.90±0.73	9.95±0.22
20.0	95.12±0.17	18.27±0.62
40.0	95.33±0.11	58.98±0.70
60.0	95.63±0.21	80.51±0.53

The results are means of three replicates ± standard deviation

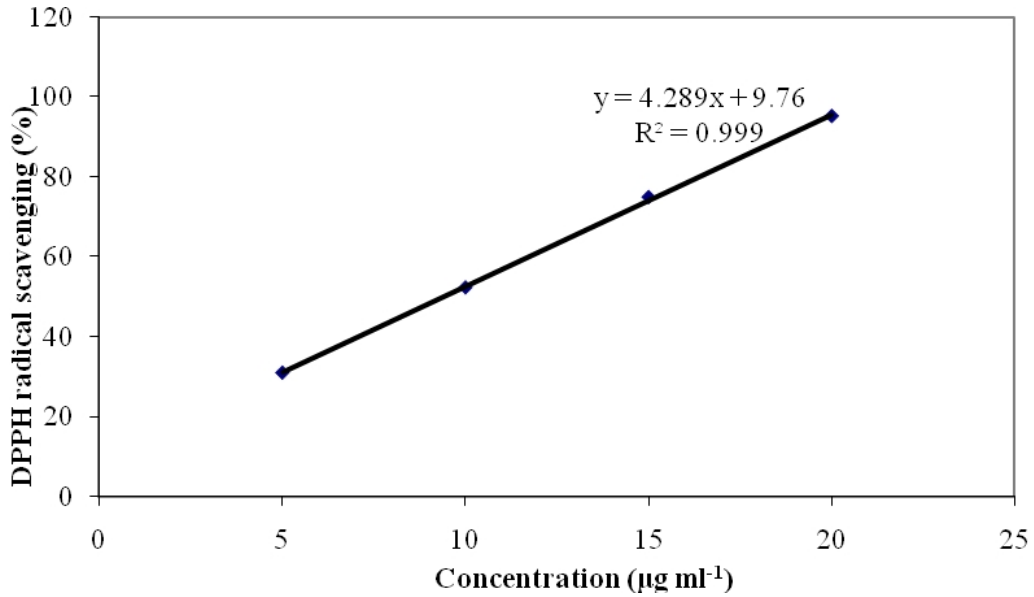


Fig. 2. Antioxidant capacity (DPPH radical scavenging activity) of gallic acid isolated from *L. abyssinica* n-butanol fraction)

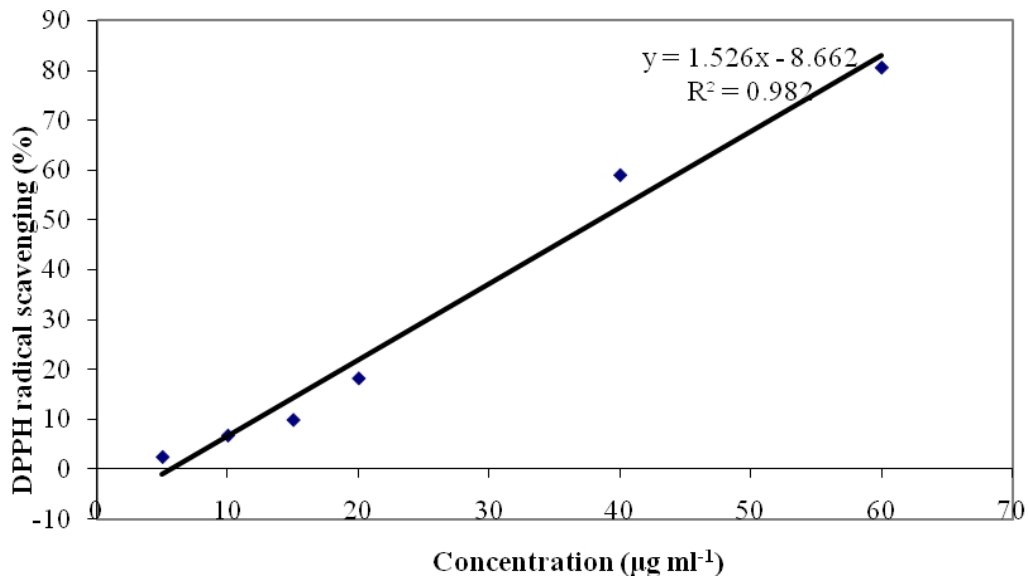


Fig. 3. Antioxidant capacity (DPPH radical scavenging activity) of standard compound (ascorbic acid)

4. DISCUSSION

The discovery of antibiotics in the course of human history led to the control or eradication of infections that hitherto ravaged mankind. However, there is increasing trend in the

development of resistance against the existing antimicrobial agents by infectious microorganisms [6]. This has resulted in interests in the exploration of plants used in traditional medicines as potential sources of novel antimicrobial compounds. Natural products and their derivatives have historically been invaluable as a source of therapeutic agents [34]. Interests in natural therapies and increasing demand for effective, safe and natural products therefore means that quantitative data on plant extracts are required.

Various parts of *L. abyssinica* A. Rich. are traditionally used as herbal remedies [23]. Amongst others, it has been used for the treatment of ailments such as cough, skin diseases, sores, diarrhea, dysentery, cholera and generalized pain [20,23]. Stem and root extracts of the plant are applied orally for the treatment of snake bites/poisoning by ethnic groups from Southern Ethiopia [35]. The whole or parts of the plant are also consumed for nutritional purposes [20,25,36]. Though several workers have reported the antimicrobial activities of several species of the genus *Ludwigia* [37,38,39], this is the first time, to our knowledge, that the antimicrobial and antioxidant effects of isolated chemical constituent of *Ludwigia abyssinica* are being reported.

The phenolic compound identified as 3,4,5-trihydroxybenzoic acid, also known as gallic acid, was isolated through bioguided fractionation of *n*-butanol extracts of *L. abyssinica* leaf. The compound showed antimicrobial effects which compared favorably with those of reference drugs - streptomycin and amphotericin B. It exhibited broad spectrum inhibitory effects on both Gram positive and Gram negative bacteria, and all the tested fungal species. Shikimic acid (3,4,5-trihydroxycyclohexene carboxylic acid) was isolated as the major compound from another specie of the genus - *Ludwigia alternaria* Linn, also found to have antimicrobial properties [40]. Shikimic acid has been found to be the starting point in the biosynthesis of some phenolics including gallic acid [41,42]. Gallic acid had been isolated from several other plant species and reported to exhibit considerable antimicrobial activities [43,44,45].

In this study, Gram positive bacteria such as *S. aureus*, *B. subtilis* and *Cl. sporogenes* were observed to show better susceptibility to gallic acid compared to the Gram negative bacteria such as *E. coli* and *P. aeruginosa*. Gram negative species are reported to be more resistant to inhibition by most plant extracts due to their outer membrane [46,47]. The presence of certain enzymes in the periplasmic space of Gram negative bacteria which are capable of breaking down the molecules introduced from outside has also been offered as an explanation [48].

Free radical scavenging potential of an antioxidant is thought to be a result of its hydrogen donating ability [49]. The scavenging activity of gallic acid at 95.63±0.21% was comparable to that of ascorbic acid (80.51±0.53%) used as standard drug at the same concentration of 60 µg ml⁻¹. This indicates that the compound possesses high DPPH scavenging activity at the highest concentration (60 µg ml⁻¹) or has high proton donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. The results strongly suggest that phenolics are important components of *L. abyssinica*, and some of its pharmacological effects could be attributed to these constituents. Gallic acid isolated from several other plant species were found to exhibit potent antioxidant activities [50,51,52]. Plant phenolics are major compounds acting as primary antioxidants or free radical terminators [53,54]. The radical scavenging abilities of these compounds are mainly due to the presence of hydroxyl groups and their redox properties [55,56]. These properties play important roles in the ability of phenolic compounds to absorb and neutralize free radicals, quench active oxygen species and decompose peroxides [55,57]. The isolated phenolic compound would therefore be expected to have high antioxidant capacities. The prevention

of risks of oxidative stress-associated diseases such as cardiovascular and neurodegenerative diseases, cancer or osteoporosis has been partially ascribed to phenolic compounds [58]. This has resulted in respective nutritional recommendations.

Preliminary phytochemical screening of this plant revealed the presence of the polyphenolic compound tannin [26]. Gallic acid is an essential precursor for many plant secondary metabolites, particularly hydrolysable tannins such as gallotannins and ellagitannins which is also a primary anti-inflammatory and cardio-protective agent found in wine, tea and cocoa [59]. Extracts from the different members of the Onagraceae family were reported by researchers to have diverse biological effects and these properties are ascribed to the high content of polyphenols, such as tannins [60,61]. Tannins provide wide range of anti-infective activities by interfering with the normal physiological characteristics of microorganisms [62]. The antimicrobial mechanisms of tannins include metal ions deprivation and complexation with enzymes or substrates leading to enzyme inhibition and substrate deprivation [63]. Some groups of tannins have been found to be inhibitory to the growth of intestinal bacteria such as *Bacteroides fragilis*, *Costridium perfringens*, *Escherichia coli* and *Enterobater cloacae* amongst others, which justify the ethnomedicinal use of several plants containing them, in the treatment of various abdominal disorders [64,65]. Tannins are also reported to be either bacteriostatic or bactericidal on *Staphylococcus aureus* [63]. Potential synergistic action with β -lactam group of antibiotics in the treatment of diseases caused by multiresistant *S. aureus* could therefore be considered.

This study has to some extent validated the medicinal potentials of *L. abyssinica* A. Rich. The isolated gallic acid may be useful as active ingredient in antimicrobial drug formulations, or as agent for the control of free radical-related pathological damages.

5. CONCLUSION

Our results indicate that gallic acid isolated from the *n*-butanol fraction of *L. abyssinica* A. Rich leaves presented *in vitro* antibacterial and antifungal activities which compared favorably with those of reference drugs used. This polyphenolic compound exhibited broad spectrum antimicrobial activities against all test bacterial and fungal species. The compound also exhibited strong antioxidant activity based on DPPH radical scavenging capacity. The plant could therefore be a potential source of natural antimicrobial and antioxidant agents for the treatment of microbial infections and prevention of various oxidative stress-associated diseases, such as cancer and other degenerative human diseases.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999;12(4):564-82. PMID: 10515903 PMCID:PMC88925
2. Rodriguez-Fragoso L, Reyes-Esparza J, Burchiel S, Herrera-Ruiz D, and Torres E. Risks and benefits of commonly used herbal medicines in Mexico. Toxicol Appl Pharmacol. 2008;227(1):125-35.
Available: <http://dx.doi.org/10.1016/j.taap.2007.10.005>
PMid: 18037151 PMCID:PMC2322858
3. Sofowora AE. Medicinal plants and traditional medicines in Africa. 2nd ed. Ibadan, Nigeria: Spectrum Books. 1993;289.
4. Reynolds JEF. Martindale - Extra Pharmacopoeia 31st ed. London: Royal Pharmaceutical Society of Great Britain; 1996.
5. Singer ME, Harding I, Jacobs MR, Dena HJ. Impact of antimicrobial resistance in the out-patient treatment of adult community-acquired pneumonia: a probability model. J Antimicrob Chemother. 2003;51:1269-82.
Available: <http://dx.doi.org/10.1093/jac/dkg220> PMid: 12697646.
6. Okeke IN, Abiodun OA, Byarugaba DK, Ojo KK, Opintan JA. Growing problem of multidrug-resistant enteric pathogens in Africa. Emerg. Infect Dis. 2007;13(11):1640-46. Available: <http://dx.doi.org/10.3201/eid1311.070674>
PMid: 18217545 PMCID:PMC3375797.
7. Gupta SS. Prospects and perspectives on natural plant products in medicine. Indian J Pharmacol. 1994;26:1-2.
8. Dahiru D, William ET, Nadroi MS. Protective effects of *Ziziphus mauritania* leaf extract on carbon tetrachloride-induced liver injury. Afr J Biotechnol. 2005;4(10): 1177-79.
9. Wilcox JK, Ash SL, Catignani GL. Antioxidants and prevention of chronic disease. Crit Rev Food Sci Nutr. 2004;44:275-95.
Available: <http://dx.doi.org/10.1080/10408690490468489> PMid: 15462130.
10. Ivanova D, Gerova D, Chervenkov T, Yankova T. Polyphenols and antioxidant capacity of Bulgarian medicinal plants. J Ethnopharmacol. 2005;96:145-50.
Available: <http://dx.doi.org/10.1016/j.jep.2004.08.033> PMid: 15588663.
11. Katalini V, Milos M, Kulisic T, Jukic M. Screening of 70 medicinal plant extracts for antioxidant capacity and phenols. Food Chem. 2006;94:550-57.
Available: <http://dx.doi.org/10.1016/j.foodchem.2004.12.004>
12. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. Int J Biomed Sci. 2008;4:89-96. PMID: 23675073 PMCID:PMC3614697.
13. Manach C, Scalbert A, Morand C, Remesy C, Jimenez L. Polyphenols: food sources and Bioavailability. Am J Clin Nutr. 2004;79:727-47. PMID: 15113710.
14. Singleton VL. Naturally occurring food toxicants: phenolic substances of plant origin common in foods. Adv Food Res. 1981;27:149-242.
Available: [http://dx.doi.org/10.1016/S0065-2628\(08\)60299-2](http://dx.doi.org/10.1016/S0065-2628(08)60299-2)
15. Krogh R, Yunes R. Structure-activity relationships for the analgesic activity of gallic acid derivatives. Farmaco. 2000;55:730-35.
Available: [http://dx.doi.org/10.1016/S0014-827X\(00\)00094-X](http://dx.doi.org/10.1016/S0014-827X(00)00094-X)

16. Stich HF, Rosin MP, Brison L. Inhibition of mutagenicity of a model nitrosation reaction by naturally occurring phenolics, coffee and tea. *Mutat Res.* 1982;95:119-28.
Available: [http://dx.doi.org/10.1016/0027-5107\(82\)90251-2](http://dx.doi.org/10.1016/0027-5107(82)90251-2)
17. Gali HU, Perchellet EM, Klish DS, Johnson JM, Perchellet JP. Antitumor-promoting activities of hydrolysable tannins in mouse skin. *Carcinogenesis.* 1992;13:715-18.
Available: <http://dx.doi.org/10.1093/carcin/13.4.715> PMID: 1576722.
18. Zhongbing L, Guangjun N, Peter S, Belton C, Huiru T. Structure-activity relationship analysis of antioxidant ability and neuroprotective effect of gallic acid derivatives. *Neurochem Int.* 2006;48:263-74.
Available: <http://dx.doi.org/10.1016/j.neuint.2005.10.010> PMID: 16343693.
19. Chen CJ, Hoch PC, Raven PH. Systematics of *Epilobium* (Onagraceae) in China. *Systematic Bot Monogr.* 1992;34:1-209.
Available: <http://dx.doi.org/10.2307/25027806>
20. Van der Burg WJ. *Ludwigia abyssinica* A. Rich. In: Grubben GJH, Denton OA, editors. *PROTA 2: Vegetables/Legumes. [CD-Rom]. PROTA*, Wageningen, Netherlands [Online]; 2004. Accessed 26 July 2012.
Available: http://database.prota.org.org/PROTAhtml/Ludwigia%20abyssinica_En.htm
21. Ruaux B, Sabine G, Jacques H, Jean-Pierre B. Sexual reproduction of two alien invasive *Ludwigia* (Onagraceae) on the middle Loire River, France. *Aquatic Bot.* 2009;90:143-48.
Available: <http://dx.doi.org/10.1016/j.aquabot.2008.08.003>
22. Burkill HM. *The Useful Plants of West Tropical Africa.* 2nd ed. Vol. 4, Families M-R. Kew, Richmond, United Kingdom: Royal Botanical; 1997;303-08.
23. Baerts M, Lehmann J. Healers and medicinal plants of the region ridges Zaire-Nile in Burundi. Royal Museum for Central Africa, Tervuren, Belgium. *Ann Sc Eco* 18 (218) From the databank PHARMEL 2 (ref. HP 10). *Prelude Medicinal Plants Database* [Online]; 1989. Accessed 26 July 2012.
Available: http://www.africamuseum.be/collections/external/prelude/view_plant/Ludwigia_abyssinica.
24. Mainen JM, Otieno DF, Mbabazi PK, Weisheit A. The ethnobotany of the Haya people of Bugabo ward, Kagera region, Northwestern Tanzania. *J Ethnobiol Ethnomed.* 2009;5:24-8.
Available: <http://dx.doi.org/10.1186/1746-4269-5-24>
PMid: 19715617 PMCID:PMC2739164
25. Igoli JO, Ogaji OG, Tor-Anyiin TA, Igoli NP. Traditional medicine practice amongst the Igede people of Nigeria. Part II. *Afr J Tradit Complement Altern Med.* 2005;(2):134-52.
26. Oyedeji O, Oziegbe M, Taiwo FO. Antibacterial, antifungal and phytochemical analysis of crude extracts from the leaves of *Ludwigia abyssinica* A. Rich. and *Ludwigia decurrens* Walter. *J Med Plant Res.* 2011;5(7):1192-99.
27. Assam Assam JP, Dzoyem JP, Pieme CA, Penlap VB. *In vitro* antibacterial activity and acute toxicity studies of aqueous-methanol extract of *Sida rhombifolia* Linn (Malvaceae). *BMC Comp Alt Med.* 2010;10:40.
Available: <http://dx.doi.org/10.1186/1472-6882-10-40>
PMid: 20663208 PMCID:PMC2922083
28. Ezoubeiri A, Gadhi CA, Fdil N, Benharref A, Jan M, Vanhaelen M, Isolation and antimicrobial activity of two phenolic compounds from *Pulicaria odora* L. *J Ethnopharmacol.* 2005;99:287-92.
Available: <http://dx.doi.org/10.1016/j.jep.2005.02.015> PMID: 15894140.
29. Mothana RA, Lindequist U. Antimicrobial activity of some medicinal plants of the island of Soqotra. *J Ethnopharmacol.* 2005;96:177-81.
Available: <http://dx.doi.org/10.1016/j.jep.2004.09.006> PMID: 15588668.

30. Clinical Laboratory Standards Institute (CLSI). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically: Approved Standard, 8th ed. Wayne, PA: M07-A8. CLSI; 2009.
31. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Medica*. 1998;64:711-13. Available: <http://dx.doi.org/10.1055/s-2006-957563> PMID: 9933989.
32. Koksall E, Bursal E, Dikici E, Tozoglu F, Gulcin I. Antioxidant activity of *Melissa officinalis* leaves. *J Med Plant Res*. 2011;5(2):217-22.
33. Gülçin I, Beydemir S, Alici HA, Elmasta_M M, Büyükkuroglu ME. *In vitro* antioxidant properties of morphine. *Pharmacol Res*. 2004;49:59-66. Available: <http://dx.doi.org/10.1016/j.phrs.2003.07.012> PMID: 14597153.
34. Mitscher LA, Drake S, Gollapudi SR, Okwute SK. A modern look at folkloric use of anti-infective agents. *J Natl Products*. 1987;50:1025-40. Available: <http://dx.doi.org/10.1021/np50054a003> PMID: 3443855.
35. Tolossa K, Debela E, Athanasiadou S, Tolera A, Ganga G, Houdijk JGM. Ethno-medicinal study of plants used for treatment of human and livestock ailments by traditional healers in South Omo, Southern Ethiopia. *J Ethnobiol Ethnomed*. 2013;9:32. Available: <http://dx.doi.org/10.1186/1746-4269-9-32> PMID: 23680260 PMCID: PMC3679849.
36. Abulude FO. Nutritional evaluation of aquatic weeds in Nigeria. *Electronic J Environ Agric Food Chem*. 2005;4:835-40.
37. Ahmed F, Selim MST, Shilpi JA. Antibacterial activity of *Ludwigia adscendens*. *Fitoterapia*. 2005;76:473-75. Available: <http://dx.doi.org/10.1016/j.fitote.2005.04.007> PMID: 15964155.
38. Das B, Kundu J, Bachar SC, Uddin MA, Kundu JK. Antitumor and antibacterial activity of ethyl acetate extract of *Ludwigia hyssopifolia* Linn and its active principle piperine. *Pakistan J Pharm Sci*. 2007;20(2):128-31. PMID: 17416568.
39. Aliyu AB, Musa AM, Abdullahi MS, Oyewale AO. Phytochemical and antibacterial properties of *Ludwigia suffruticosa* (Wild) Iliv. Ex. O. Ktze (Onagraceae). *Int J Pure Appl Sci*. 2008;2(4):1-5.
40. Rao AS, Merugu R. Shikimic acid as a major compound from *Ludwigia alternifolia* Linn. *J Pharmacognosy* 2012;3(2):121-122.
41. Werner RA, Rossmann A, Schwarz C, Bacher A, Schmidt HL, Eisenreich W. Biosynthesis of gallic acid in *Rhus typhina*: discrimination between alternative pathways from natural oxygen isotope abundance. *Phytochemistry*. 2004;65(20):2809-13. Available: <http://dx.doi.org/10.1016/j.phytochem.2004.08.020> PMID: 15474568.
42. Muir RM, Ibariez AM, Uratsu SL, Ingham ES, Leslie CA, McGranahan GH. Mechanism of gallic acid biosynthesis in bacteria (*Escherichia coli*) and walnuts (*Juglans regia*). *Plant Mol Biol*; 2011. PMID: 21279669.
43. Pretto JB, Cechinel-Filho V, Noldin VF, Sartori MRK, Isaias DEB, Cruz AB. Antimicrobial Activity of Fractions and Compounds from *Calophyllum brasiliense* (Clusiaceae/Guttiferae). *Z Naturforsch*. 2004;59:657-662.
44. Li A, Chen J, Zhu W, Jiang T, Zhang X, Gu Q. Antibacterial activity of gallic acid from the flowers of *Rosa chinensis* Jacq. against fish pathogens. *Aquaculture Res*. 2007;38(10):1110-12. Available: <http://dx.doi.org/10.1111/j.1365-2109.2007.01745.x>
45. Chanwitheesuk A, Teerawutgulrag A, Kilburn JD, Rakariyatham N. Antibacterial activity of gallic acid from *Caesalpinia mimosoides* Lamk. *Food Chem*. 2007;100(3):1044-48. Available: <http://dx.doi.org/10.1016/j.foodchem.2005.11.008>

46. Longbottom CJ, Carson CF, Hammer KA, Mee BJ, Riley TV. Tolerance of *Pseudomonas aeruginosa* to *Melaleuca alternifolia* (tea tree) oil is associated with the outer membrane and energy-dependent cellular processes. *J Antimicrob Chemother.* 2004;54:386-92.
Available: <http://dx.doi.org/10.1093/jac/dkh359> PMID: 15254026.
47. Nikaido H. Outer membrane. In: Neidhardt FC, editors. *Escherichia coli* and *Salmonella typhimurium*: Cellular and Molecular Biology. Washington: American Society for Microbiology. 1996;29-47.
48. Gao Y, Belkum MUV, Stiles M. The outer membrane of Gram-negative bacteria inhibits antibacterial activity of brochocin C. *Appl Environ Microbiol.* 1999;65:4329-33. PMID: 10508055 PMCID:PMC91573.
49. Baumann J, Wurn G, Bruchlausen FV. Psostaglandin synthetase inhibiting O₂ radical scavenging properties of some flavonoids and related phenolic compounds. Deutsche pharmakologische Gesellschaft Abstracts of the 20th Spring meeting, Naunyn-Schmiedebergs Abstract No. R27 cited. *Arch P Pharmacol.* 1979;307:1-7.
50. Cho YH, Kim JH, Sim GS, Lee BC, Pyo HB, Park HD. Inhibitory effects of antioxidant constituents from *Melothria heterophylla* on matrix metalloproteinase-1 expression in UVA-irradiated human dermal fibroblasts. *J Cosmet Sci.* 2006;57(4):279-89. PMID: 16957808.
51. Feng-lin H, Li-ming Y, Shwu-fen C, Li-hsuan W, Chung-yi W, Pan-chun L, Shwu-jian L. Biotransformation of gallic acid by *Beauveria sulfurescens*. *Appl Microbiol Biotechnol.* 2007;74:659-66.
52. Muhammed S, Khan BA, Akhtar N, Mahmood T, Rasul A, Hussain I, Khan H, Badshah A. The morphology, extractions, chemical constituents and uses of *Terminalia chebula*: A review. *J Med Plant Res.* 2012;6(33):4772-75.
53. Yang JH, Lin HC, Mau JL. Antioxidant properties of several commercial mushrooms. *Food Chem.* 2002;77:229-35.
Available: [http://dx.doi.org/10.1016/S0308-8146\(01\)00342-9](http://dx.doi.org/10.1016/S0308-8146(01)00342-9)
54. Milliauskas G, Venskutonis PR, Van-Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chem.* 2004;85:231-37.
Available: <http://dx.doi.org/10.1016/j.foodchem.2003.05.007>
55. Osawa T. Novel natural antioxidants for utilization in food and biological systems. In: Uritani I, Garcia VV, Mendoza EM, editors. Post harvest biochemistry of plant food-materials in the tropics. Japan: Japan Scientific Societies Press. 1994;241-51.
56. Zheng W, Wang YS. Antioxidant activity and phenolic compounds in selected herbs. *J Agric Food Chem.* 2001;49:5165-70.
Available: <http://dx.doi.org/10.1021/jf010697n> PMID: 11714298.
57. Duh PD, Tu YY, Yen GC. Antioxidant activity of water extract of Harng Jyur (*Chrysanthemum morifolium* Ramat). *Lebensm Wiss Technol.* 1999;32:269-77.
Available: <http://dx.doi.org/10.1006/fstl.1999.0548>
58. Arts IC, Hollman PC. Polyphenols and disease risk in epidemiologic studies. *Am J Clin Nutr.* 2005;81:317-25. PMID: 15640497.
59. Grundhofer P, Niemetz R, Schilling G, Gross GG. Biosynthesis of subcellular distribution of hydrolysable tannins. *Phytochemistry.* 2001;57(6):915-27.
Available: [http://dx.doi.org/10.1016/S0031-9422\(01\)00099.1](http://dx.doi.org/10.1016/S0031-9422(01)00099.1) PMID: 11423141.
60. Kiss AK, Bazylko A, Filipek A, et al. Oenothien B's contribution to the anti-inflammatory and antioxidant activity of *Epilobium* sp. *Phytomedicine.* 2011;18(7):557-60.
Available: <http://dx.doi.org/10.1016/j.phymed.2010.10.016> PMID: 21112753.

61. Paredes CC, Balbas PB, Gomez-Velasco A, Juarez ZN, Arreola ES, Hernandez LR, Bach H. Antimicrobial, antiparasitic, anti-inflammatory and cytotoxic activities of *Lopezia racemosa*. The Scientific World Journal. 2013;2013:1-6.
Available: <http://dx.doi.org/10.1155/2013/237438> PMID: 23843731 PMCID:PMC3694551
62. Okwu DE, Okwu ME. Chemical composition of *Spondias mombin* Linn. plant parts. J Sustain Agric Environ. 2004;6(2):140-47.
63. Chung KT, Wong TY, Wei CY, Huang YW, Lin Y. Tannins and human health: A review. Crit Rev Food Sci Nutr. 2008;38:421-64.
Available: <http://dx.doi.org/10.1080/10408699891274273> PMID: 9759559.
64. Akiyama H, Fujii K, Yamasaki O, Oono T, Iwatsuki K. Antibacterial action of several tannins against *Staphylococcus aureus*. J. Antimicrob Chemother. 2001;48(4):487-91.
Available: <http://dx.doi.org/10.1093/jac/48.4.487> PMID: 11581226.
65. Machado TD, Leal ICR, Amaral ACF, dos Santos KRN, da Silva MG, Kuster RM. Antimicrobial ellagitannin of *Punica granatum* fruits. J Braz Chem Soc. 2002;13:606-10.
Available:
<http://dx.doi.org/10.1590/S0103-50532002000500010>
<http://dx.doi.org/10.1590/S0103-50532002000500030>

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