Chemistry Africa (2023) 6:845–853
https://doi.org/10.1007/s42250-022-00535-w

ORIGINAL ARTICLE

Antimicrobial Activity and Phytochemical Constituents of Leaf Extracts of *Englerina woodfordioides* (Schweinf.) M. Gilbert

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Received: 13 September 2022 / Accepted: 2 November 2022 / Published online: 19 November 2022

Abstract

Englerina woodfordioides is a hemi-parasitic shrub known for its various ethnomedical uses. Nonetheless, these alleged therapeutic benefits are not scientifically validated. In addition, the study of its phytochemical composition remains unexplored. This study aimed at investigating the antimicrobial activity of *E. woodfordioides* collected from four different host plants. The antibacterial activities and toxicity of crude leaf extract were evaluated using the agar disk diffusion assay and animal model, respectively. The phytochemical composition of active leaf extracts was determined using LC-MS Q-TOF analysis. The crude chloroform, ethyl acetate, and aqueous extracts of *E. woodfordioides* did not inhibit the growth of *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* at 100 mg/ml. Conversely, the methanolic extracts of *E. woodfordioides* inhibited antibacterial effects against *S. aureus.* The acute toxicity test using dry methanol extract revealed no signs of toxicity or death of white mice at doses of 175, 550, and 2000 mg/kg body weight. Thirty-four and eleven metabolites were detected in the methanolic extract of *E. woodfordioides* collected from *Schinus molle* and *Vachellia abyssinica* host plants, respectively. The presence of flavonoids, phenolic acids, and alkaloids may be responsible for the antimicrobial activity of *E. woodfordioides*. This is the first comprehensive study on the antimicrobial activity, toxicity, and phytochemical constituents of *E. woodfordioides* collected from different host plants. Further study may reveal the potent bioactive principles with antimicrobial properties.

Keywords *Englerina woodfordioides* · Metabolomics · Antimicrobial activity · Acute toxicity · Ethiopia

Abbreviations

ANOVA	Analysis of variance
DMSO	Dimethyl sulfoxide
E. coli	*Escherichia coli*
EPHI	Ethiopian Public Health Institute
ETH	National Herbarium at Addis Ababa University, Ethiopia
EWDP	*Englerina woodfordioides* collected from *Discopedium penninervum*
EWEG	*Englerina woodfordioides* collected from *Eucalyptus globulus* host plant
EWSM	*Englerina woodfordioides* collected from *Schinus molle* host plant
EWVA	*Englerina woodfordioides* collected from and *Vachellia abyssinica*
INT	p-Iodonitrotetrazolium dye
MBC	Minimum bactericidal concentration
MHA	Muller Hinton Agar
MIC	Minimum inhibitory concentration
OECD	Organization for Economic Cooperation and Development guideline
P. aeruginosa	*Pseudomonas aeruginosa*
LC-MS Q-TOF/MS	Liquid chromatograph mass spectroscopy quadrupole time-of-flight mass spectrometry
ESI	Electrospray ionization

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1 Introduction

Natural products deriving from medicinal plants are exploited in the traditional medicinal systems of many communities around the world. About 80% of the world's population living in developing countries uses traditional medicine to address their primary healthcare needs [1]. Various secondary metabolites in these plants serve as source of novel molecules for drug discoveries [2, 3]. Meanwhile, the pharmacological activities of several species of medicinal plants in Africa have not been validated scientifically for their alleged health benefits [3]. In recent decades, there has been a growing interest in exploring medicinal plants to overcome the rapid emergence of antibiotic-resistant microorganisms [4, 5].

The genus *Englerina* belongs to the family Loranthaceae. All 25 species of the genus are grown in tropical Africa and are hosted by 19 families of plant species on the continent [6]. *Englerina woodfordioides* (syn. *Loranthus woodfordioides*) is a shrub that grows on a wide range of host plants [7–9]. It is distributed in Burundi, Ethiopia, Kenya, Rwanda, Tanzania, Uganda, and Zaire [7, 10]. In the traditional medicine of Ethiopia, *E. woodfordioides* has been utilized for the treatment of earaches [11], wounds [12], cutaneous leishmaniasis [13, 14], syphilis [15], and malaria [16]. In the southern region of Ethiopia, the therapeutic importance of *E. woodfordioides* has been observed to vary depending on the host plant on which it grows [9].

The scientific study on the medicinal properties of Loranthaceae species has attracted less attention in Ethiopia. This is mainly associated with the recurrent removal of these plants because of their devastating effect on the survival of host plants, the uneven distribution of species, the difficulty of propagating them, and the lack of enough samples for study. As a result, there is no literature on the biological activity and identity of chemical compounds of Loranthance species, including *E. woodfordioides*, in Ethiopia. Therefore, the present study was designed to investigate the antimicrobial activity, acute toxicity, and phytochemical constituents of the leaf extracts of *E. woodfordioides* in Ethiopia.

2 Materials and Methods

2.1 Collection and Authentication of Plant Samples

The leaves of *E. woodfordiodes* were collected from Shashemene, Gambo, and Menagesha localities in Central Ethiopia between April and June 2018. These samples were harvested from *Shinus molle*, *Discopodium penninervum*, *Eucalyptus globulus*, and *Vachellia abyssinica*, hereafter designated as EWSM, EWDP, EWEG, and EWVA, respectively. Taxonomic identification of these specimens was done by Melaku Wondafrash Herbarium Specialist at the National Herbarium (ETH) at Addis Ababa University, Ethiopia. These specimens were given the voucher numbers (AY 2, AY 19, AY 4, and AY 34 for EWSM, EWDP, EWEG, and EWVA, respectively) and deposited at ETH.

2.2 Material Processing and Extraction

Healthy leaves were washed under running tap water. These leaves were air-dried for 7 days and pulverized using a Retsch electrical grinding mill (Hulme-Martin Ltd, UK). Twenty (20) grams of each sample specimen's were macerated in 200 ml of analytical grade chloroform, ethyl acetate, methanol, and sterilized distilled water in a separate glass jar for 72 h. The extracts were filtered using Whatman® no1 filter paper (Maidstone, England). These filtrates were concentrated under reduced pressure in a RE-100D rotary evaporator (Phoenix Instrument) at 40 °C for 45 min for organic solvents, and using a lyophilizer (CHRIST, Germany) for aqueous extracts.

2.3 Antimicrobial Activity

The antibacterial activities of *E. woodfordiodes* extracts were evaluated against *Escherichia coli* ATCC 25,922, *Pseudomonas aeruginosa* ATTC 27,853, and *Staphylococcus aureus* ATCC 25,923 provided by the Ethiopian Public Health Institute (EPHI), Addis Ababa, Ethiopia. Disk diffusion method was used for the screening of antimicrobial activity of each specimens extracts. The broth cultures of these bacteria were diluted with 0.9% (w/v) sterilized normal saline solution from an overnight grown colonies. The bacterial suspensions were adjusted to 0.5 McFarland turbidity [DEN-1 (BioSan, Latvia)] and spread on Muller Hinton Agar (MHA) growth medium using a sterile cotton swab. The extracts were diluted in dimethyl sulfoxide (DMSO). Sterile filter paper disks (Ø = 6 mm) loaded with 20 µL of the test extracts (100 mg extract dissolved in 1mL DMSO) were placed over the MHA medium. Similarly, disks impregnated with DMSO and the four extraction solvents were prepared to serve as negative controls and placed over the MHA medium. The standard disks of Amoxycillin/Clavulanic acid (30 g for *E. coli* and *S. aureus*), Ampicillin (10 g for *E. coli* and *P. aeruginosa*), Erythromycin (10 g for *S. aureus*), Gentamycin (10 g for *P. aeruginosa* and *E. coli*), and Tetracycline (30 g, for *E. coli*) were used as positive controls. The inoculated plates were incubated for 16 to 18 h at 35 ± 2°C. The diameter of the inhibition zone was measured using a transparent ruler and reported in mm, including

the 6 mm paper disk. Plant extracts having an apparently higher zone of inhibition were selected for further study.

2.4 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC and MBC of plant extracts were determined using a rapid *p*-Indonitrotetrazolium chloride (INT; ROTH, Carl Roth GmbH Co., Germany) colorimetric assay by the broth macrodilution method as described by [17, 18] with slight modifications. Briefly, 0.5 mL of the stock solution (100 mg of crude plant extract dissolved in 1 mL of 99.9% DMSO) was drawn and diluted with 0.5 mL of 2% DMSO to minimize the toxicity effect of DMSO [19, 20]. The resulting solution was diluted in a two-fold dilution to a range of 50–0.098 mg/mL in 10 separate test tubes. One milliliter of plant extract solution was discarded from the last test tube. Each of these solutions was transferred into 10 test tubes that were filled with 2 ml Muller-Hinton broth (MHB, sterilized broth at 121°C, 15 Pa for 15 min). Test tube 11 (no extract added, but with 1 mL of 2% DMSO) and test tube 12 (no extract but with 1 mL of 2% DMSO and 60 µL of 30 mg/ml erythromycin) served as a negative or growth control and a positive control, respectively. Bacterial suspension (100 µL) was added to each of these test tubes. Then, 60 µL of INT (2 mg/ml of INT in sterile distilled water) was added to each of these test tubes to assess the viability of the bacterium after 18 h of incubation [21]. These test tubes were further incubated for 2 h at 35 ± 2°C to determine the MIC of extracts, which is the lowest concentration of plant extract that inhibited bacterial growth [22]. This is evidenced by the absence of pink coloration of the dye [21]. Finally, the MBC of plant extracts was determined after determination of the MIC of the test plant extracts. Aliquots of 100 µl of each gradient concentrated solution were transferred into new test tube filled with fresh MHB, and incubated at 35 ± 2°C for 48 h. The MBC endpoint was considered as the lowest concentration of plant extract that showed no color change after the addition of INT [23].

2.5 Acute Toxicity Test

Adult healthy female white mice (21.15 ± 2.11 g, 8–12 weeks old) were purchased from the EPHI. These mice were acclimatized for five days in a polypropylene cage (5 mice each) before dosing. Mice were supplied with animal food and water for free access. They were kept at 22 ± 3 °C and on a 12 h light/dark cycle in the Animal House of the College of Natural and Computational Sciences of Addis Ababa University.

The acute oral toxicity test was conducted as described by the Economic Cooperation and Development guideline [24]. The dry crude extract of *E. woodfordioides* with apparent antimicrobial activity was simultaneously administered to three (3) groups of five (5) mice each, namely 175 mg/kg, 550, and 2000 mg/kg body weight doses. The positive and negative control groups of mice (5 mice each) were administered with normal saline and tap water, respectively. Each mouse was observed for signs of toxicity during the first half an hour, after 4 and 24 h, and subsequently daily for 14 days. The body weight of an individual mouse was determined shortly before the commencement of dosing and afterwards in the first and second week of the dosing period. The relative organ weight (ROW) of the lungs, heart, kidneys, and liver of an individual mouse was weighed after the end of the acute oral toxicity test. The percentage weight gain [25] and relative organ [26] weight of mice were calculated using the following formulae.

$$\text{Percentage weight gain} = (\text{Final weight} - \text{Initial weight})/\text{Initial weight} \times 100.$$

$$\text{ROW} = (\text{Absolute organ weight (g)}/\text{Body weight on sacrifice day (g)}) \times 100.$$

2.6 Silica Gel Fractions Activity

The crude extracts of EWSM and EWVA with apparent antimicrobial activity were further fractionated using a silica gel column (60 g) eluted with n-hexane, n-hexane/chloroform, chloroform, chloroform/ethyl acetate, ethyl acetate, ethyl acetate/methanol, and methanol. All sub-fractions were tested for their antimicrobial activity as mentioned above.

2.7 Phytochemical Analysis of Metabolites Using LC-MS Q-TOF Analysis

Metabolomic analyses of the crude extracts were done using liquid chromatography-mass spectrometry (LC-MS) analysis on Agilent HP 1260 Infinity Series Liquid Chromatograph equipped with a DAD system (Agilent Technologies system, Santa Clara, CA, USA) coupled to MS Q-TOF model G6540B (Agilent Technologies). The C-18 column (Adamas® 4.6 × 50 mm, 3.5 µm, SepaChrom Srl, Rho, Mi, Italy) was maintained at a constant temperature of 25°C, and used as the chromatography separations column. The mobile phase consisted of 0.1% (*v/v*) formic acid in water (phase A) and 0.1% (*v/v*) formic acid in acetonitrile (phase B). The analyses were performed at a flow-rate of 0.5 mL min^{-1}, 95% A graduating to 70% B in 4 min, 100% B 4–5 min, graduating from 70 to 80% B in 3 min, graduating from 80% B to 100% B in 2 min and equilibrating at 95% A 10–14 min. The UV spectra were collected by DAD every 0.4 s from 190

Table 1 Diameter of inhibition zones in mm (mean ± SD) of methanolic extracts

Study plants	Control	Test concentrations (mg/mL)		
	Erythromycin	100	50	25
EWSM	35.000 ± 0.000	14.667 ± 0.153	13.667 ± 0.153	6.00 ± 0.000
EWEG	28.667 ± 0.058	7.333 ± 0.058	6.00 ± 0.000	6.00 ± 0.000
EWDP	28.333 ± 0.058	10.667 ± 0.058	6.00 ± 0.000	6.00 ± 0.000
EWVA	27.667 ± 0.115	19.000 ± 0.265	14.333 ± 0.153	13.333 ± 0.153

to 750 nm with a resolution of 2 nm. The mass spectrometer system was equipped with a DUAL ESI ionization source that was set in both positive and negative mode. Mass spectra were recorded from *m/z* 100 to 1700, with 3 scans per second. The optimized conditions were: nebulizer pressure 11psig; desolvation gas temperature, 350°C; desolvation gas flow, 11 L/min and capillary voltage, 2 kV, fragmentor at 180 V, cone 1 (skimmer 1) at 45 V, Oct RFV at 750 V. A 10 µL of sample was injected into the system. Raw data were evaluated based on retention time and characteristic behavior of MS, including the exact mass, quasimolecular ions and in-source fragmentation, using Mass Hunter Qualitative Analysis Software version B.06.00 (Agilent Technologies). These data were compared with known compounds in an in-house plant database and existing literature [27]. Positive identifications of plant metabolites were considered for analysis if the compound was detected with a mass error below 10 ppm and with a sufficient score.

2.8 Statistical Analysis

All results on the antimicrobial assay and acute toxicity test were carried out by one-way analysis of variance (ANOVA) using the SAS version 9.4 software package. The mean ± standard deviation was used to express these data. Tukey's test was used to calculate a statistically significant difference among treatments. All results were considered statistically significant at $p \leq 0.05$.

3 Results

3.1 Antimicrobial Activity

The crude methanolic extracts of EWSM, EWEG, EWDP, and EWVA inhibited the growth of *S. aureus* at 100 mg/ml of crude extracts. At this concentration, the crude methanol extracts of EWSM and EWVA showed a higher zone of inhibition compared with EWEG and EWDP at 100 and 50 mg/ml (Table 1, Supplementary file Figs. 1, 2, 3 and 4). In contrast, the crude extracts of ethyl acetate, chloroform, and aqueous solvents did not inhibit the growth of *S. aureus*. In addition, the crude ethyl acetate, chloroform, aqueous, and methanol extracts of these four specimens have shown no activity against *E. coli* and *P. aeruginosa.* The standard antibiotic disks have shown a higher zone of inhibition than all crude extracts. Consequently, further investigation into the antimicrobial activities, MIC, MBC, acute toxicity and phytochemical composition of *E. woodfordioides* was conducted using the crude methanol extracts of EWSM and EWVA.

The MIC for EWSM and EWVA against *S. aureus* was observed at 25 and 12.5 mg/mL, respectively. The negative controls showed full growth of *S. aureus* at 2% DMSO. Conversely, there was no bacterial growth in erythromycin-treated test tubes. On the other hand, the MBC of EWSM and EWVA were more than 50 mg/mL against *S. aureus* (Supplementary file Figs. 5, 6, 7 and 8). The ethyl acetate and ethyl acetate: methanol fractionates of EWSM and the ethyl acetate fractions of EWVA exhibited antimicrobial activity against *S. aureus*. None of the remaining fractions showed inhibition activity against *S. aureus*.

3.2 Acute Toxicity Test

The administration of crude methanol extracts of EWSM and EWVA exhibited hair erection, hiding in the shavings of the softwood, and being sleepy during the first 4 h of observation. Then after, all mice were active and showed no sign of diarrhea, death, or toxicity throughout the 14 days of observation at all test doses. There was an overall weight gain of mice in the second week of dosing. However, these weight gains were not significantly different among the treatment and control groups of mice (Table 2).

There was a significant difference ($p < 0.05$) in the mean weight of the kidney with respect to the body weight of the mice. The mean weight of the heart, lungs, and liver did not differ significantly. An exceptional significant difference ($p > 0.05$) was observed between the body weight of the mice treated with 2000 mg/kg of plant extract and the tap water treated mice (Table 3).

3.3 Phytochemical Analysis of Metabolites Using LC-MS Q-TOF Analysis

In total, 34 and 11 metabolites were identified in the crude methanol extracts of EWSM (Table 4, Supplementary file Figs. 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20,

Table 2 Methanol extract of *E. woodfordioides* leaves on body weight gain of mice (mean ± SD)

Plant	Concentration	Body weight of mice (g)		
		Initial day	7th day	14th day
EWSM	175 mg/mL	22.140 ± 0.904	22.087 ± 2.018	25.781 ± 2.651
	550 mg/mL	20.000 ± 0.904	19.206 ± 2.018	30.229 ± 2.651
	2000 mg/mL	20.800 ± 0.904	19.625 ± 2.018	30.786 ± 2.651
	Saline	21.300 ± 0.904	13.331 ± 2.018	18.947 ± 2.651
	Water	21.200 ± 0.904	16.170 ± 2.018	29.969 ± 2.651
EWVA	175 mg/mL	21.400 ± 0.906	17.666 ± 2.176	27.900 ± 2.614
	550 mg/mL	20.000 ± 0.906	15.768 ± 2.176	22.256 ± 2.614
	2000 mg/mL	19.600 ± 0.906	16.692 ± 2.176	30.702 ± 2.614
	Saline	21.200 ± 0.906	13.870 ± 2.176	19.496 ± 2.614
	Water	21.200 ± 0.906	16.170 ± 2.176	28.386 ± 2.614

Table 3 Relative vital organ weight of female Swiss albino mice (mean ± SD, n = 5)

Specimen	Conc. (mg/kg)	Body weight (g)	Heart	Lung	Liver	Kidney
EWSM	175	29.616 ± 1.990^{b}	0.583 ± 0.061	0.756 ± 0.065	5.476 ± 0.644	1.153 ± 0.070^{a}
	550	$26.432 \pm 2.196^{a,b}$	0.614 ± 0.039	0.716 ± 0.080	5.622 ± 0.697	$1.265 \pm 0.103^{a,b}$
	2000	29.466 ± 3.292^{b}	0.574 ± 0.089	0.745 ± 0.042	6.012 ± 0.365	1.152 ± 0.130^{a}
EWVA	175	28.442 ± 1.567^{b}	0.524 ± 0.039	0.700 ± 0.078	5.059 ± 0.455	1.089 ± 0.040^{a}
	550	$25.050 \pm 0.835^{a,b}$	0.534 ± 0.040	0.785 ± 0.067	5.391 ± 0.324	$1.219 \pm 0.101^{a,b}$
	2000	23.526 ± 2.184^{a}	0.684 ± 0.193	0.869 ± 0.074	5.790 ± 0.625	1.382 ± 0.161^{b}
Saline		$25.996 \pm 2.829^{a,b}$	0.580 ± 0.082	0.779 ± 0.115	5.440 ± 0.622	$1.240 \pm 0.096^{a,b}$
Water		28.500 ± 2.114^{b}	0.563 ± 0.059	0.834 ± 0.173	5.589 ± 0.399	$1.172 \pm 0.086^{a,b}$

*Means with similar letter(s) in a column are not significantly different

21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42 and 43) and EWVA (Table 5, Supplementary file Figs. 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71 and 72), respectively. In these crude extracts, a greater number of metabolites was detected in the negative ionization mode than in the positive ionization mode. Eight metabolites were shared between the crude methanol extracts of EWSM and EWVA specimens. Flavonoids and phenolics were the most detected classes of compounds in these specimens.

In the EWSM, four compounds were identified in both positive and negative ionization modes. Six out of the nine compounds were found in both ethyl acetate-methanol fractions of EWSM (Table 6, Supplementary file Figs. 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59 and 60). Most of these compounds were detected in the positive ionization mode. In contrast, 23 compounds were identified in ethyl acetate I and II fractions of EWVA (Table 7, Supplementary file Figs. 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103 and 104). Most of the compounds in ethyl acetate-I were identified in the negative ionization mode. An equivalent number of compounds were identified in both ionization modes of ethyl acetate-II. In addition, eight metabolites were commonly shared between the ethyl acetate I and II fractionates of EWVA (Table 7). Five compounds, namely albine, hyperin, melanoxetin, reynoutrin, and trifolin were shared between the two fractions of EWSM and EWVA.

4 Discussion

The present study demonstrated that the crude methanol leaf extracts of *E. woodfordioides* inhibit the growth of *S. aureus.* The antimicrobial activity of methanol extracts over other solvent extracts may be associated with the polarity of methanol solvent and the presence of polar bioactive secondary metabolites in these specimens [28]. This is also supported by the antimicrobial activity of polar fractions (ethyl acetate-methanol and the ethyl acetate fractionates) than the low to medium polar (n-hexane, n-hexane/chloroform, chloroform, chloroform/ethyl acetate) fractions of EWSM and EWVA, respectively, against *S. aureus*. Conversely, the relatively lower inhibition zones exhibited by the methanol extracts of EWEG and EWDP may be related to the difference in the quality and quantity of bioactive compounds available in these specimens, which may partly depend on the host plant

Table 4 Phytochemical compounds identified from the crude methanol leaf extracts of EWSM using LC-MS Q-TOF analysis

Compound names	Chemical formula	Monoisotopic mass (Da)	RT (min)	Molecular ion (*m/z*)	Ionization mode
(-)-Epicatechin	$C_{15}H_{14}O_6$	290.0792	4.898	$[M-H]^-$289.072	–
(-)-Epicatechin 3-O-gallate	$C_{22}H_{18}O_{10}$	442.0903	5.287	$[M-H]^-$441.0831	–
4-Caffeoylquinic acid	$C_{16}H_{18}O_9$	354.095	4.795	$[M-H]^-$ 353.0877	–
Catechin-(4alpha->8)-epicatechin-3-O-gallate	$C_{37}H_{30}O_{16}$	730.1531	5.013	$[M-H]^-$729.1458	–
Kaempferol 7-(6''-galloylglucoside)	$C_{28}H_{24}O_{15}$	600.1113	5.213	$[M-H]^-$599.104	–
Oleuropein	$C_{25}H_{32}O_{13}$	540.1842	5.407	$[M-H]^-$539.1769	–
Orientin 2″-acetate	$C_{23}H_{22}O_{12}$	490.1112	5.289	$[M-H]^-$489.104	–
Quercetin 3-(2″-acetylgalactoside)	$C_{23}H_{22}O_{13}$	506.1064	5.078	$[M-H]^-$505.0992	–
Quinic acid	$C_7H_{12}O_6$	192.064	1.285	$[M-H]^-$191.0568	–
Robinetidinol(4alpha-6)catechin	$C_{30}H_{26}O_{12}$	578.1422	4.663	$[M-H]^-$577.1349	–
Robinetin	$C_{15}H_{10}O_7$	302.0419	5.347	$[M-H]^-$301.0346	–
Rutin	$C_{27}H_{30}O_{16}$	610.1532	5.015	$[M-H]^-$609.1459	–
Subalatin	$C_{24}H_{20}O_9$	452.1094	5.464	$[M-H]^-$451.1022	–
Trifolin	$C_{21}H_{20}O_{11}$	448.1013	5.35	$[M-H]^-$447.0941	–
(Z,Z,Z)-Octadeca-9,12,15-trienoic acid	$C_{18}H_{30}O_2$	278.2245	6.942	$[M-H]^+$279.2318	+
1-Methyl-3-(1-methylethyl)-1,2-cyclopentanedicarboxylic acid	$C_{11}H_{18}O_4$	214.1201	5.8	$[M+H\text{-}H_2O]^+$197.1168	+
2-Phenylethanol	$C_8H_{10}O$	122.0728	4.595	$[M+H\text{-}H_2O]^+$105.0695	+
Adenine	$C_5H_5N_5$	135.0545	1.358	$[M+H]^+$136.0618	+
Caffeic acid	$C_9H_8O_4$	180.0419	4.804	$[M+H\text{-}H_2O]^+$163.0387	+
Deoxy phytoprostane J1	$C_{18}H_{26}O_3$	290.1881	6.833	$[M+H]^+$291.1955	+
Fisetin	$C_{15}H_{10}O_6$	286.0477	5.499	$[M+H]^+$287.0551	+
Indole-3-acrylic acid	$C_{11}H_9NO_2$	187.0629	4.704	$[M+H]^+$188.0702	+
Indole-3-carboxaldehyde	C_9H_7NO	145.052	4.702	$[M+H]^+$146.0594	+
Indole-3-ethanol	$C_{10}H_{11}NO$	161.0833	4.702	$[M+H\text{-}H_2O]^+$144.0799	+
Melanoxetin	$C_{15}H_{10}O_7$	302.0428	5.335	$[M+H]^+$303.0501	+
Methyl isoeugenol	$C_{11}H_{14}O_2$	178.0989	5.802	$[M+H]^+$179.1062	+
Shikimic acid	$C_7H_{10}O_5$	174.0525	6.597	$[M+H\text{-}H_2O]^+$157.0492	+
Stachydrine	$C_7H_{13}NO_2$	143.0942	1.345	$[M+H]^+$144.1014	+
Trans-(+-)-2-Methyl-5-(2-methyl-5-isopropyltetrahydro-2-furyl)furan	$C_{13}H_{20}O_2$	208.1458	6.506	$[M+H]^+$209.1532	+
Trigonelline	$C_7H_7NO_2$	137.0476	1.3	138.0549	+
3-Oxo-2-(2-entenyl) cyclopentaneoctanoic acid	$C_{18}H_{30}O_3$	294.2202	8.335	$[M-H]^-$293.2129	±
Hyperin	$C_{21}H_{20}O_{12}$	464.0964	5.179	$[M-H]^-$463.0893	±
Quercetin 7-(6″-galloylglucoside)	$C_{28}H_{24}O_{16}$	616.107	5.112	$[M-H]^-$615.0999	±
Reynoutrin	$C_{20}H_{18}O_{11}$	434.0855	5.326	$[M-H]^-$433.0783	±

Ionization mode: + indicate positive, – negative, ± indicate both positive and negative ionization mode

species [29]. The sensitivity of *S. aureus* (Gram-positive) and the resistance of *E. coli* and *P. aeruginosa* (Gram-negative) bacteria with respect to the different solvent extracts of *E. woodfordioides* may be explained by the variation in the cell wall morphology and composition of the two groups of bacteria [30]. In addition, the lower inhibition zone by EWSM and EWVA compared with the standard drugs may be the result of the quality and quantity of bioactive compounds in the crude extracts of *E. woodfordioides*. Despite the fact that no previous study report is available on the acute toxicology of *E. woodfordioides* extracts, the absence of physical and behavioral changes, and the death of mice after the administration of 2000 mg/kg bw may indicate the low toxicity of *E. woodfordiodes* as described in the Globally Harmonized System of Classification and Labeling of Chemicals [31]. Meanwhile, the mean weight of the kidney may be due to other intrinsic factors of the extract.

Previous studies on the phytochemical composition of *E. woodfordioides* reported the presence of flavonoids, quinones, terpenoids, steroids [32], and condensed

Table 5 List of phytochemical compounds identified from the crude leaf extracts of EWVA by LC-MS Q-TOF

Compound names	Chemical formula	Monoisotopic mass (Da)	RT (min)	Molecular ion (*m/z*)	Ionization mode
(-)-Epicatechin 3-O-gallate	$C_{22}H_{18}O_{10}$	442.0905	5.25	$[M-H]^-$441.0833	–
3-Oxo-2-(2-entenyl)cyclopenta-neoctanoic acid	$C_{18}H_{30}O_3$	294.2205	10.008	$[M-H]^-$293.2133	–
Gallic acid	$C_7H_6O_5$	170.0215	4.69	$[M-H]^-$169.0142	–
Hyperin	$C_{21}H_{20}O_{12}$	464.096	5.155	$[M-H]^-$463.0887	–
Quinic acid	$C_7H_{12}O_6$	192.064	1.28	$[M-H]^-$191.0567	–
Reynoutrin	$C_{20}H_{18}O_{11}$	434.0856	5.332	$[M-H]^-$433.0784	–
Robinetin	$C_{15}H_{10}O_7$	302.0435	6.05	$[M-H]^-$301.0363	–
Trifolin	$C_{21}H_{20}O_{11}$	448.101	5.334	$[M-H]^-$447.0938	–
Erythritol	$C_4H_{10}O_4$	122.0581	1.836	$[M+H-H_2O]^+$105.0548	+
Isorhamnetin	$C_{16}H_{12}O_7$	316.0583	5.53	$[M+H]^+$317.0656	+
Melanoxetin	$C_{15}H_{10}O_7$	302.0413	5.332	$[M+H]^+$303.0486	+

Ionization mode: + indicate positive, – negative, ± indicate both positive and negative ionization mode

Table 6 Metabolites identified from the fractions of crude methanol extracts of EWSM with apparent antimicrobial activity by LC-MS Q-TOF

Compounds	Ethyl acetate-methanol I	Ethyl acetate-methanol II
Adenine	√	
Albine	√	√
Fisetin	√	√
Hyperin	√	√
Melanoxetin	√	√
Quercetin 7-(6’’-galloylglucoside)		√
Reynoutrin	√	√
Succinic acid		√
Trifolin	√	√

tannins [33]. There were no alkaloids and tannins [32]. Nonetheless, these studies did not report details of these phytochemicals. On the other hand, the presence of 4-hydroxybenzoic acid [34], erythritol [35], isorhamnetin [36], fisetin and hyperin [37], and succinic acid [38] were reported for the antimicrobial activity of these metabolites against *S. aureus*. In agreement with these studies, the detection of various secondary metabolites such as flavonoids and phenolic acids in the leaves of EWSM and EWVA may be responsible for the antimicrobial activity of these plant extract against *S. aureus*. The detection of different metabolites between the crude extracts and fractionates of EWSM and EWVA might be associated with the variation in the extraction process and polarities of chemical compounds used during the extraction of the plant [28, 39].

Table 7 List of phytochemical compounds identified from the bioactive fractionates of the leaves of EWVA by LC-MS Q-TOF

Compound names	Ethyl acetate I	Ethyl acetate II
4-hydroxybenzoic acid	√	
8-Hydroxyfustin	√	
Albine		√
D-(+)-Galactose	√	√
Erythritol	√	√
Hyperin	√	√
Isorhamnetin	√	√
Isorhamnetin 3-galactoside	√	
Kaempferol 3-neohesperidoside	√	
Ligstroside	√	
Maltose	√	
Melanoxetin	√	√
Methyl benzoate	√	
Methyl salicylate	√	
Oleuropein	√	√
Ononitol	√	
Orientin 2’’-acetate	√	
Quinic acid	√	
Reynoutrin	√	√
Robinetin	√	
Rutin	√	
Trifolin	√	√
Tyramine	√	

5 Conclusion

The crude methanol leaf extract of *E. woodfordioides* has antimicrobial activity against *S. aureus* with no prolonged signs of toxicity and mortality in mice. Accordingly, this plant is safe and non-toxic as stated in the Globally

Harmonized System of Classification and Labeling of Chemicals. This is the first study to identify metabolites from the leaves of *E. woodfordioides*. Therefore, it is necessary to further isolate pure compounds responsible for the antimicrobial activity of the plant.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42250-022-00535-w.

Acknowledgements We thank Addis Ababa University (AAU) and the EPHI for providing technical support and bacterial strains. AY is grateful to Marlies Spohn, Helmut Spohn and his colleagues at Ethiopian Forest Development, Central Ethiopia Center and AAU for their unreserved assistance and encouragement. We thank Dr Adey Feleke for her motivation during laboratory works and financial assistance for laboratory consumables. Finally, our thanks go to the editor of the Journal and the anonymous reviewer for their critical comments and suggestions to improve the manuscript.

Funding Partial financial support was received from Addis Ababa University that covers fieldwork expenses and purchase of some laboratory consumables through thematic projects.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval and consent to participate The study was carried out following all relevant guidelines and regulations.

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