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Original Article

Antibacterial Potential and Safety Level of *Euphorbia tirucalli* and *Vernonia glabra* Commonly used by Residents in Iringa, Tanzania

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ABSTRACT

This study was conducted to evaluate the antibacterial potential of *Euphorbia tirucalli* L. and *Vernonia glabra* (Steetz) Vatke and their safety level. The plants were obtained from Kiwere ward in the Iringa district, prepared and extracted in aqueous and ethanol solvents. The *E. tirucalli* and *V. glabra* plant extracts were studied for their antimicrobial activities against *E. coli* isolates and *E. coli* ATCC 25922 using the agar well diffusion method. Minimum inhibitory and bactericidal concentrations (MIC and MBC) were determined. Among the tested plant extracts, the ethanol extracts of *V. glabra* showed the highest activity against *E. coli* isolates and *E. coli* 25922 with a mean inhibition zone of 17.909±0.3297 and 22.5 ± 0.500 respectively. Furthermore, on MIC and MBC, the ethanol extracts of *V. glabra* had a better activity with the values ranging from 1.25-2.5 mg/ml and 2.5-5 mg/ml respectively compared to other plant extracts. Phytochemical screening revealed the extract's presence of alkaloids, flavonoids, tannins, saponins, triterpenes and steroids. The brine shrimp lethality test showed that the aqueous extract of *E. tirucalli* was non-toxic with LC_{50} of 1007 µg/mL, while ethanol extract of *E. tirucalli*, aqueous extract of *V. glabra* and ethanol extract of *V. glabra* were less toxic with LC_{50} values of 589, 507 and 658 µg/mL respectively. These results indicate that the plant extracts have bioactive constituents that could be accounted for their pharmacological properties. Furthermore, these results proved the claim of indigenous people in the study area on the effectiveness and safety of the plants and supported the use of these plant species in traditional medicine.

INTRODUCTION

Medicinal plants are a great source of antimicrobial compounds in various types of plants [1]. They have been used for centuries in different parts of the world, for prevention and curative for both humans and animals [2]. The antimicrobial activities of many plants are attributed to the presence of secondary metabolites [3]. Therefore, various research is being conducted worldwide to evaluate these plants biological constituents and therapeutic values [4]. In the global population, about 87.5% use traditional herbal medicine to treat health problems [5]. Similarly, about 60-85% of the population in developing countries relies on traditional medicine for their primary healthcare needs due to its accessibility and affordability [6]. The increase in microbial infections and the development of antimicrobial resistance (AMR) to synthetic drugs have also led researchers to pay attention to investigating plants as natural sources of biological substances against microorganisms of medical and veterinary importance [7–9].

The present study focused on investigating the antimicrobial potential of *Euphorbia tirucalli* and *Vernonia glabra* the plants used by different people in the world as well as Tanzania in the treatment of infectious diseases in humans and animals. *E. tirucalli* L. belongs to the family *Euphorbiaceae*, the species are native to Africa and are widely distributed in semi-arid areas of the tropical and most sub-tropical regions [10, 11]. The plant is a valuable source of medicinal compounds such as alkaloids,

tannins, flavonoids, terpenoids, saponins and phenols which contributed to their effectiveness in medicinal treatment [12, 14]. The different parts of the plant such as the latex, leaves, stems and roots may have different medicinal properties [12]. Although, latex has many medicinal potentials, it is considered toxic if consumed in excess and is reported to cause conjunctivitis when it accidentally gets in contact with the eyes [15, 16].

V. glabra belongs to the family *Asteraceae*, is an herbaceous perennial plant with flowers [17] and are widely found in the tropics, subtropics and temperate regions [18,19]. Previous studies revealed that the genus *Vernonia* has different phytochemicals of medicinal properties which justify their use in traditional herbal medicine to treat microbial-based diseases [20]. Also, the species have ethno medicine use for managing several diseases and used as food in some African regions [21].

In Tanzania, specifically, the Iringa district, *V. glabra* is used as a medicinal plant for animals and humans. Traditionally *V. glabra* is commonly used for the treatment of gastrointestinal diseases, headaches, malaria, urinary tract infection, infertility, cough, colds, and sexually transmitted diseases for humans [21–23]. Also, the plant is used by the smallholder farmers in Iringa for prevention, control and treatment of different clinical conditions in animals especially in chickens. The most common symptoms, clinical signs and diseases treated with *V. glabra* when observed in chicken or flocks were yellowish-colored droppings, watery and or bloody diarrhea, serious nasal discharges, depression and respiratory distress. This claim indicates that ethnoveterinary medicine is of great importance to the rural people and smallholder farmers, as it is easily accessible, apparently effective and cheap.

In this study, *Escherichia coli (E. coli)* was isolated from chickens and tested to evaluate the effectiveness of these medicinal plants. *E. coli* is used as a representative organism since it is abundant in the digestive system of warm-blooded animals, and it has been extensively used to monitor AMR in food animals including chicken[24,25].Also, its susceptibility patterns reflect the diversity of resistance in a bacterial population [26]. *E. coli* is the major cause of avian colibacillosis, a serious infectious disease in poultry [27], and also causes disease conditions in chickens such as yolk sac infections, pericarditis, peritonitis and osteomyelitis [28]. In addition, some *E. coli* strains hosted by poultry are potential sources of AMR genes that may transmit to humans [29]. Therefore, the findings from this study would be an essential step for these plants to be used as a source of antibiotic agents as an alternative approach for treating diseases caused by resistant *E. coli*.

MATERIALS AND METHODS

Plant Materials Collection

The fresh stem of *E. tirucalli* and leaves of *V. glabra* were collected at Kiwere Ward (7°64'388''S, 35°62'096"E) on January 2022 in the rural district of Iringa Region, Tanzania. The botanist from Mkwawa University College of Education at Iringa identified the plants and voucher specimens (KW-ET001, KW-VG001) were prepared and deposited at the University herbarium. The samples were placed in a plastic bag special for plant sample storage and transported to the Sokoine University of Agriculture, College of Veterinary Medicine and Biomedical Sciences, Department of Veterinary Physiology, Biochemistry and Pharmacology for further process.

Source of Chemicals, Solvents, Reagents and Media

All chemicals used in this study were of analytical grade, and purchased from university suppliers. The chemicals were from Loba Chemie Pvt Ltd, Mumbai, INDIA. Dimethyl sulfoxide (DMSO) was from RFCL Limited, Hayana, India. All media and Gentamycin drugs were supplied by OXOID®, Basingstoke, UK. The brine shrimps (*Artemia salina*) eggs and sea salt were obtained in the Department of Chemistry and Physics, Sokoine University of Agriculture-Tanzania.

Preparation of Plant Extract

The leaves of *V. glabra and* stems of *E. tirucalli* collected were separately cleaned and air dried at room temperature for five days and fourteen days respectively. *E. tirucalli* was also dried in a hot air oven at 40 ºC for three days. The dried leaves and pieces of the stem were separately ground into a fine powder using a laboratory mill and obtained 264.4g of *E. tirucalli* and 255g of *V. glabra* which were stored in airtight bags.

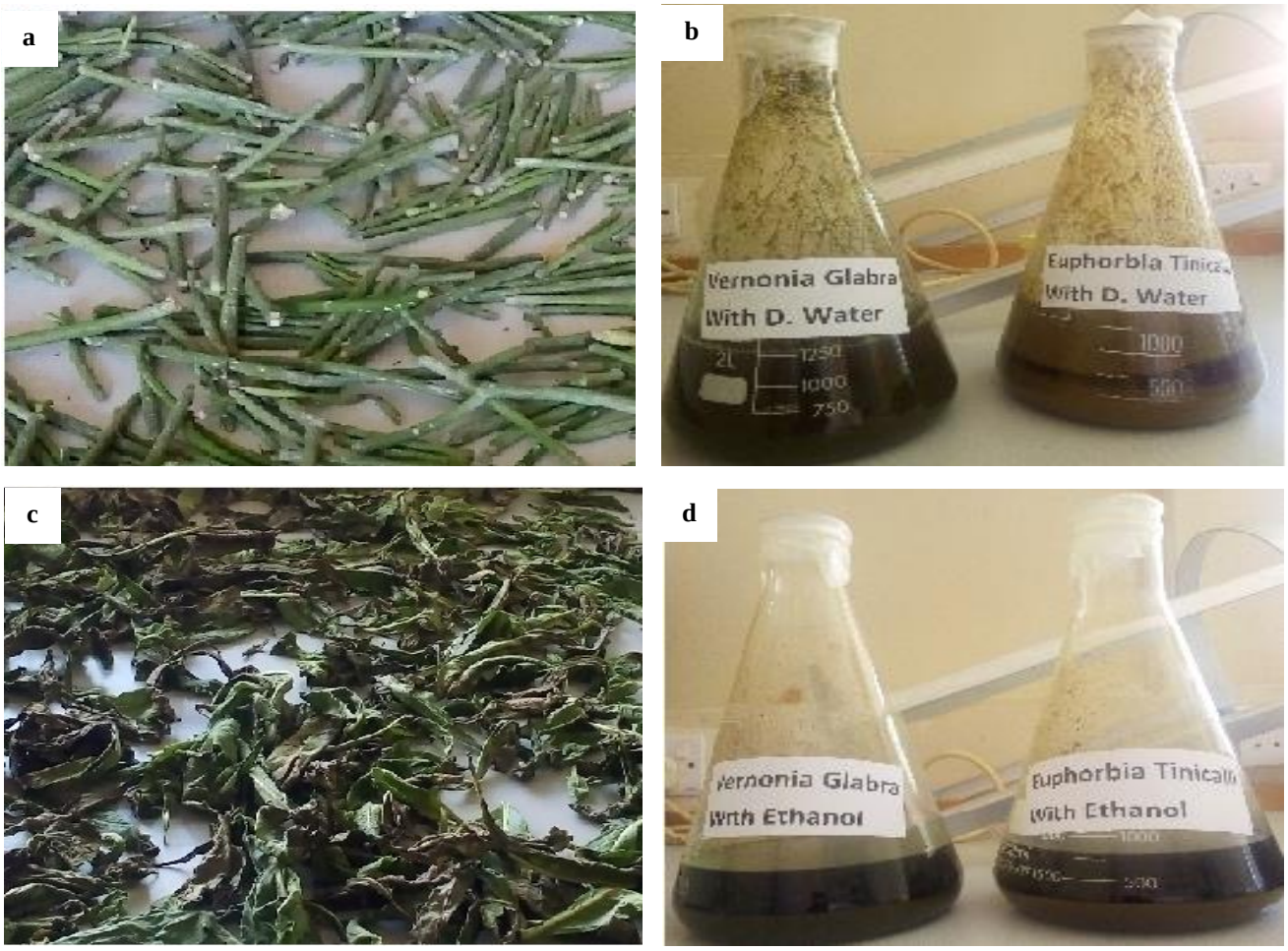

Fig. 1 Source: Experimental photo from the laboratory, a: Dried stems of *E. tirucalli*, b: Samples soaked in water, c: Dried leaves of *V. glabra*, d: Samples soaked in ethanol

Aqueous and Ethanol Extraction

The extracts of the plant materials were obtained using maceration methods. Briefly, the aqueous extracts were prepared by mixing 125 g of each sample with 1250 ml of distilled water (1:10 sample to solvent ratio). The ethanol extracts were prepared by dissolving 125 g of each sample in 625 ml of 80% ethanol (1:5 sample to solvents ratio). The mixtures were left to soak for 72 hours at room temperature. Then the mixture was filtered using a filter funnel fitted with gauze and cotton wool. The procedure was repeated two times to obtain sufficient crude extracts. Water was evaporated using a water bath for three days and ethanol extracts were concentrated on water bath overnight both at 50 °C. The dried extracts were transferred to sample bottles and stored in refrigerator at 4 °C for further use. From crude extracts, working solutions of 20, 40, 60 and 80 mg/ml were prepared using 10% DMSO.

Source of Bacteria

The bacteria used for bioassay was *E. coli* non-clinical isolates and ATCC 25922. The *E. coli* isolates were obtained from layer chicken cloaca in poultry farms in Morogoro (Wanna *et al*, unpublished). Bacterial identification was performed through macromorphological, micromorphological and biochemical profiling. The antibiotic susceptibility testing using commercial drugs such as; ampicillin, tetracycline, sulfamethoxazole/ trimethoprim, ciprofloxacin, cefotaxime and gentamicin was done to obtain the resistance profile of the isolates. *E. coli* ATCC 25922 was used as a standard control strain and it was obtained at the Microbiology laboratory at Sokoine University of Agriculture (SUA). All isolates were sub cultured onto nutrient agar to ensure purity and viability.

Inoculum Preparation

E. coli suspensions were prepared by picking two to three isolated colonies from a 24-hour-old culture

plate using a sterile wire loop and inoculating them into 5ml normal saline. The inoculum was thoroughly mixed by vortex mixer and the turbidity of the suspension was adjusted by comparing it to 0.5 McFarland standard.

Screening for the Antibacterial Potential of the Plant Extracts

Agar Well Diffusion Method

The evaluation of antibacterial activity was carried out by agar well diffusion method as described by Valgas *et al.,* [30] with some modifications. The Standardized *E. coli* inoculum was uniformly spread using a sterile cotton swab on the entire surface of a sterile petri dish in Muller Hinton Agar (MHA) and dried for 5 minutes. A sterile cork borer of 6 mm diameter was used to punch four holes which represented concentrations of 20, 40, 60, and 80 mg/ml along with positive and negative control. On each concentration, 50µL of respective plant extract was added to each of the four wells. Gentamycin disc (10µg) was used as a positive control and 10% DMSO was used as a negative control.

The inoculated Petri dishes were left for a few minutes for the extract to diffuse into an agar plate and then incubated at 37 °C for 18-24 hours, under aerobic conditions. The diameter zone of complete inhibition of each well and disk was measured using a ruler in (mm) at the back of the petri dish in reflecting light and recorded. Each test was performed in triplicate.

Determination of Minimum Inhibitory Concentration (MIC) for Plant Extract

Minimum inhibitory concentration (MIC) was done by using the two-fold serial microdilution method [31] in a sterile 96-well plate. The plant extracts 20mg/ ml from both samples labeled samples A-D were tested against *E. coli* isolates and *E. coli* ATCC 25922. Muller Hinton Broth (MHB) which was prepared according to manufacturer protocol, gentamicin as a standard antibiotic and sterile normal saline as negative control were used. MIC values were detected by observing turbidity in wells, the presence of turbid indicating growth while the absence of turbid indicating inhibition of microbial growth. The lowest concentration of plant extract at which no turbidity was observed was recorded as MIC.

Determination of Minimum Bactericidal Concentration (MBC)

The MBC was determined by taking 50 µl of the test dilutions from the microtiter plates in three wells with no visible growth in MIC assay and sub-cultured on freshly prepared MHA plates and incubated for 18–24 hours. The highest dilution that yielded no single bacterial colony on the plates was recorded as MBC.

Phytochemical Screening

The qualitative phytochemical analysis was done using standard procedures for qualitative tests for the solvent extracts with some modifications [32–35]. The extracts were analyzed for the presence of Alkaloids, Flavonoids, Saponins, Steroids, Tannins and triterpenoids.

Brine Shrimp Lethality Test (BSLT)

BSLT was used to determine if the plant extracts of medicinal species were cytotoxic [36]. Brine shrimp (*Artemia salina*) eggs were hatched in 3.4 g/l concentration of seawater on a proper petri dish with a lid, and incubated for 24 hours at room temperature. After incubation ten brine shrimp larvae (naupii) were collected by tits pipette and placed into 24 wells of microplates. Prepared plant extracts were diluted at the concentration of 1000, 500, 250, 125, 100 and 50 µg/ml using DMSO and were added in each well in duplicate. Then, seawater was added to make a total volume of 6 ml. Also, 6mls DMSO in duplicate was used as a control. The plates were covered and incubated at room temperature for 24 hours. The number of survived nauplii for each vial was counted to obtain the number of dead nauplii, and their mean at each concentration was determined. Mean percentage of mortality at each concentration was determined using the equation as described by Meyer *et al.,* [36]:

$$\text{Percentage of mortality} = \frac{\text{no.of dead naupii}}{\text{initial no.of live naupii added in a vial}} \times 100$$

Data Analysis

Statistical analysis of the data was performed using Microsoft excel® 2019 and IBM SPSS Statistics 25 software. All data for antimicrobial susceptibility were presented as mean values ± mean standard errors (SME) for the triplicate set of experiments in each case. An independent sample t-test was used for antimicrobial data in comparing means among the

plant extracts and solvents used. Analysis of variance (ANOVA) was used in comparing means between different concentrations. *P* values < 0.05 were considered significant.

The concentration killing fifty percent of the larva (LC_{50}) was calculated by using the regression line obtained by plotting the mean percentage mortality (on a probit scale) against the logarithm of concentration. The results were interpreted as follows: LC_{50} greater than 1000 µg/ml as non-toxic, LC_{50} of 500-1000 µg/ml as low toxic, LC_{50} of 100-500 µg/ml as moderate toxic, and LC_{50} of 0-100 µg/ml as highly toxic [36, 37].

RESULTS

Antibacterial Activity by Agar Well Diffusion Method

E. tirucalli and *V. glabra* extracts in different concentrations showed inhibition zone against tested *E. coli* isolates which indicated that the plant species have medicinal activities (Table 1 & 2). When compared to the plant extracts, the ethanol extracts of *V. glabra* showed the highest antibacterial activity on *E. coli* isolates and *E. coli* ATCC 25922 strain with the mean inhibition zone (in mm) ranging from 11.5 ± 0.6 to 18.0 ± 0.3 and 16.0 ± 0.0 to 22.5 ± 0.5 respectively. The aqueous extracts of *E. tirucalli* showed the lowest antimicrobial activities on *E. coli* isolates and *E. coli* ATCC 25922 strain with mean inhibition zone (in mm) ranging from 10.0 ± 0.8 to 16.6 ± 0.2 and 15.0 ± 0.0 to 19.0 ± 0.5 respectively.

The antibacterial performance of the two plant extracts was not significantly different. However, variation in terms of performance based on concentration differences in each plant extract existed ($P < 0.05$).

Gentamicin used as a positive control had an inhibition zone (in mm) ranging from 20.0 ± 0.0 to 22.5 ± 0.5 against *E. coli* ATCC 25922 and 15.3 ± 0.2 to 15.6 ± 0.2 against *E. coli* isolates. The DMSO used as a negative control did not show any zone of inhibition, indicating no antibacterial activity (Table 1 & 2).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC and MBC results are indicated in Table 3. The ethanol extract of *V. glabra* had MIC values ranging from 2.5 to 1.25 mg/ml and MBC ranging from 2.5 to 5 mg/ml against *E. coli* ATCC 25922 strains.

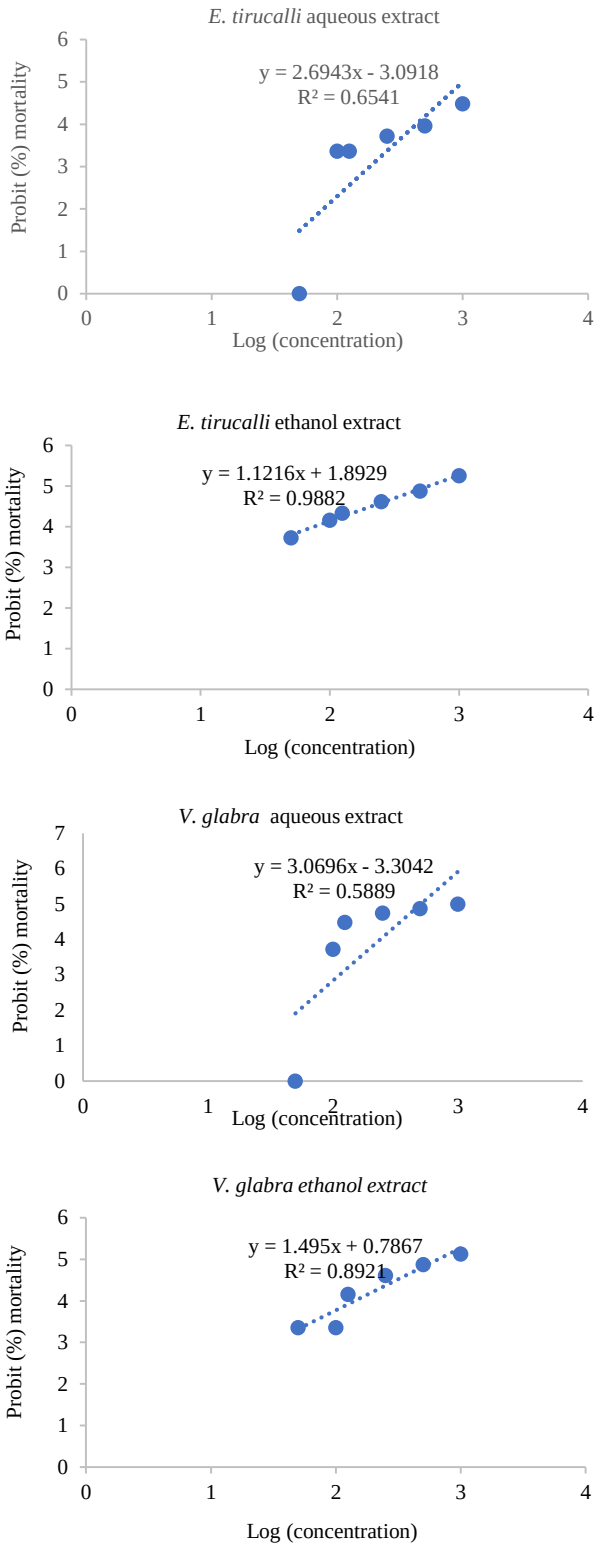

Fig. 2 Graph relationship of log concentration of (a) aqueous extract of *E. tirucalli*, (b) ethanol extract *of E. tirucalli*, (c) aqueous of *V. glabra* and (d) ethanol extract of *V. glabra* against the response of mortality of *A. salina* larvae.

The other plant extract had MIC values of 10 mg/ml and did not exhibit the bactericidal activities at the

tested concentration range in both tested organisms; *E. coli* ATCC 25922 strain and the non-clinical isolates. The standard gentamycin used as a positive control showed the highest antimicrobial activities compared with the plant extracts with the MIC values ranging from 0.625 to 0.3125 mg/ml and MBC value of 1.25 mg/ml against *E. coli* ATCC 25922 strain. Similarly, MIC values ranged from 2.5 mg/ml to 1.25 mg/ml and MBC value of 5 mg/ml against *E. coli* clinical isolates were recorded for gentamycin (Table 3).

Qualitative Phytochemical Analysis of Plant Extracts

The phytochemical screening of *E. tirucalli* and *V. glabra* for secondary metabolites revealed the presence of alkaloids, flavonoids, steroids, saponins and triterpenoids in aqueous extracts and the presence of alkaloids, flavonoids steroids and tannins in ethanol extracts (Table 4).

Brine Shrimp Lethality Test

The results revealed that the higher the concentration of the test solution, the higher the percentage of mortality of *A. salina* larvae.

In this study, the aqueous extract of *E. tirucalli* had an LC_{50} value of 1007 µg/ml which indicated that it belongs to a non-toxic class. Additionally, the LC_{50} of *E. tirucalli* in ethanol extract, *V. glabra* in aqueous and *V. glabra* in ethanol were 589 µg/ml, 507 µg/ml and 658 µg/ml indicating that they belong to the less toxic class (Table 5, and Fig 2).

Table 1 Mean zone of inhibition (in mm) of ethanol and aqueous stem extracts of *E. tirucalli*.

Extracts	Concentrations	*E. coli* isolates	*E. coli* ATCC 25922
Ethanol	20	11.3±0.5	15.5±0.5
	40	12.0±0.0	16.0±0.0
	60	15.9±0.3	21.5±0.5
	80	17.0±0.30	22.0±0.0
	CN (µg/ml)	15.3±0.2	20.5±0.5
	DMSO	0.0±0.0	0.0±0.0
Aqueous	20	10.0±0.7	15.0±0.0
	40	10.5±0.5	15.5±0.5
	60	14.8±0.2	18.0±0.0
	80	16.6± 0.2	19.0±0.5
	CN (µg/ml)	15.3±0.2	20.0±0.0
	DMSO	0.0±0.0	0.0±0.0

Table 2 Mean zone of inhibition (in mm) of ethanol and aqueous leaf extracts of *V. glabra*.

Extracts	Concentration	*E. coli* isolates	*E. coli* ATCC 25922
Ethanol	20	11.5±0.6	16.0±0.0
	40	12.5±0.3	17.0±0.0
	60	16.4±0.3	21.0±0.0
	80	18.0±0.3	22.5±0.5
	CN (µg/ml)	15.5±0.2	22.5±0.5
	DMSO	0.0±0.0	0.0±0.0
Aqueous	20	10.3±0.5	16.0±0.0
	40	10.8±0.5	16.0±0.0
	60	14.8±0.2	17.0±1.0
	80	16.8±0.4	19.5±1.0
	CN (µg/ml)	15.6±0.2	21.0±1.0
	DMSO	0.0±0.0	0.0±0.0

Table 3 Minimum inhibitory concentration (MIC) of *E. tirucalli* and *V. glabra* extracts in mg/ml

Test organisms	*E. tirucalli* in aqueous		*E. tirucalli* in ethanol		*V. glabra* in aqueous		*V. glabra* in ethanol		Gentamicin	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. coli isolates	10	-	10	-	10	-	10	-	2.5-1.25	5-2.5
E. coli ATCC 25922	10	-	10	-	10	-	2.5-1.25	5-2.5	0.625-0.3125	1.25

Table 4 Qualitative phytochemical analysis for *E. tirucalli* and *V. glabra* extracts

Plant Extracts	*E. tirucalli* in aqueous	*E. tirucalli* in ethanol	*V. glabra* in aqueous	*V. glabra* in ethanol
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Saponin	+	-	+	-
Terpenoids	+	-	+	-
Steroids	+	+	+	+
Tannins	-	+	-	+

Key: + = present of constituents, - = absence of constituents

Table 5 Brine shrimp lethality test results

		Sample A		Sample B		Sample C		Sample D	
CONC. µg/ml	LOG(Conc)	% Mortality	Probit	% Mortality	Probit	% Mortality	Probit	% Mortality	Probit
50	1.69897	0	0	10	3.72	0	0	5	3.36
100	2	5	3.36	20	4.16	10	3.72	5	3.36
125	2.09691	5	3.36	25	4.33	30	4.48	20	4.16
250	2.39794	10	3.72	35	4.61	40	4.75	35	4.61
500	2.69897	15	3.96	45	4.87	45	4.87	45	4.87
1000	3	30	4.48	60	5.25	50	5	55	5.13
-	-	LC50 = 1007.63		LC50 = 589.0606		LC50 = 507.341		LC50 = 658.036	

KEY: Sample A = *E. tirucalli* extracted in water, Sample B = *E. tirucalli* extracted in Ethanol, Sample C = *V. glabra* extracted in water, and sample D = *V. glabra* extracted in ethanol. All tests were done in duplicates and the average number of live nauplii was calculated.

DISCUSSION

Plants produce a wide range of secondary metabolites with significant structural and functional diversity, giving them a plentiful source of novel biologically active substances [38]. In the present study, phytochemical screening of aqueous and ethanol extracts revealed the presence of alkaloids, steroids, flavonoids, triterpenoids, saponins and tannins. These findings support the use of *E. tirucalli* and *V. glabra* for the treatment of different human and veterinary diseases. Various studies have recommended that the healing property of medicinal plants mainly are contributed by the presence of flavonoids, alkaloids, steroids, tannins, saponins and terpenoids [6, 39–40]. The presence and significance of phytochemical constituents on *E. tirucalli* and *V. glabra* in this study were consistence with other previous studies [12,17,20, 41-42].

Previous studies on phytochemical screening and in vitro antibacterial activity of different solvent extracts revealed that alkaloids have some protective role in plants thus why being employed in the production of various medicines [43]. Flavonoids are found to be effective antimicrobial substances against a wide range of microorganisms, this could be due to their ability to complex with extracellular and soluble proteins [44]. Saponins are known for their medicinal properties as a natural blood cleanser, expectorant and antibiotics [7]. Tannins are identified as natural polyphenolic substances that precipitate proteins [45], and also form complexes with proteins, starch, cellulose and minerals [46], resulting in the inhibition of cellular protein synthesis [6]. These secondary metabolites also are produced to help the plants to overcome stressful conditions like salinity, cold, frost, and elevated temperatures and to fight against pathogens that affect plant growth and development [47].

The antimicrobial susceptibility findings in this study showed that the studied plant extracts have

antibacterial activity. Our results support the previous findings of the antibacterial activity of *E. tirucalli* and *V. glabra* [12,17,20; 48–50] . The plants (*V. glabra* and *E. tirucalli)* vary in their activities when aqueous and ethanol extracts are used. The ethanol extract has the highest antimicrobial activity than the aqueous extracts, however, no significant differences were observed between them. The difference in antibacterial performance between ethanol and aqueous plant extract could be explained by differences in the polarity of the active ingredients in the plants being extracted by the solvents and their ability to dissolve or diffuse in water and ethanol [40, 51]. These findings agreed with the results reported by Upadhyay *et al*., [12], Ngonda *et al*., [17] and Kitonde *et al*., [20] who observed high and moderate activity of organic and aqueous extracts of stem and leaf of *E. tirucalli and V. glabra*.

Both plant extracts showed activities in varying concentrations tested with different inhibition zone values evidenced by high performance as concentration increases [42]. The variation in antibacterial performance was statistically tested to be significant. Thus, our findings support the use of *E. tirucalli* and *V. glabra* for the treatment of diseases with a potential of *E. coli* origin. The inhibitory and bactericidal effect of these extracts proves their use by the native community such as those in Iringa and the plants are potential for drug development for the treatment of infections caused by these microbes not only in humans but also veterinary side, where antimicrobial resistance takes a face of a silent pandemic.

BSLT is a rapid, cheap and simple method for screening the toxicity of bioactive plant extracts, which significantly correlates with cytotoxic and antitumor properties [36,52]. The results of this study indicated that the toxicity of plant extracts was dose-dependent, the higher the dose the higher the percentage mortality. The aqueous extract of *E. tirucalli* is non-toxic at high concentrations with LC_{50} of 1007 µg/ml. This result, is similar to the finding reported by Mackeen *et al*., [53] that *E. tirucalli* was non-toxic with $LC_{50} > 1000$. The ethanol extracts of *E. tirucalli*, aqueous extract of *V. glabra* and ethanol extract of *V. glabra* are less toxic at high concentrations with LC_{50} values of 589, 507, 658 µg/ml respectively. The finding on ethanol extract of *V. glabra* is consistent with the result reported by Nondo *et al*., [22]. The aqueous extracts were demonstrated to be safe compared to ethanol extracts. Therefore, the traditional methods of preparation are taken into consideration, because most medications are prepared as simple water extracts, thus avoiding potential toxic effects.

These findings verified that the plant extracts contained active compounds which possess high biological activities and are safe at lower concentrations, therefore have the potential to be used as antimicrobial drugs.

CONCLUSION

The present study revealed that the plant extracts have bioactive constituents that could be accounted for their pharmacological properties. Furthermore, these results proved the claim of indigenous people in the study area on its effectiveness and support the uses of these plant species in traditional medicine. Also, the study verified that the plants are safe at recommended lower doses. Because the crude plant extracts are efficacious at high concentrations and toxic at the same level of concentration. Therefore, further studies in these two plants especially in vivo studies using animal models with several multiple lower doses are essential to be done to measure microbial clearance.

Ethical Approval

The permission to carry out this study was approved by the Sokoine University of Agriculture under its Ethical Committees and given approval ref no SUA/DRPTC/R/186. The permission was also granted by the Morogoro Municipal Livestock Officer while verbal consent was obtained from each of the farmers before sampling their chickens.

Declarations

Author Contribution Statement

Devotha Wanna: Developed the idea, designed the study, performed the experiment and drafted the manuscript.

Elisa Mwega and Alexanda Mzula: supervision of the experiment and proofreading the manuscript.

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Competing Interest Statement

The authors declare no conflict of interest.

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