ISSN: 0067-2904

Phytochemical Screening and *in Vitro* Antimicrobial Activities of *Euphorbia Lateriflora* on Selected Pathogens

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Received: 1/8/2021 Accepted: 17/10/2021 Published: 30\4\2022

Abstract

Euphorbia lateriflora is a popular traditional medicinal plant whose leaves are used in Africa, especially Nigeria, to treat wounds and many diseases. This study investigated the preliminary phytochemical constituents, secondary metabolites by High-Performance Liquid Chromatography "HPLC" technique, and antimicrobial potentials (Minimum Inhibitory Concentration "MIC", Minimum Fungicidal Concentration "MFC" and disc diffusion assay) of various concentrations (100 mg/mL, 50 mg/ml, and 25 mg/mL) of the solvents (ethyl acetate and n-hexane) extracts of *E. lateriflora* against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. The phytochemical screening revealed that out of the thirteen constituents screened for, n-hexane extract contained seven components, including flavonoids, coumarin, steroid, glycosides, triterpenes, terpenoids, and alkaloids. In contrast, the ethyl acetate extract contains saponin, flavonoids, coumarin, steroid, glycosides, triterpenes, terpenoids, and alkaloids. The HPLC chromatogram of n-Hexane and ethyl acetate extracts of *E. lateriflora* detected numerous unidentified compounds along with a potential antimicrobial compound identified as caffeic acid. The low MIC (6.25 - 50 mg/mL), shown by the n-hexane extract signified a greater antimicrobial activity than ethyl acetate extract. This was further implicated by the varying concentrations levels, rather than the strain-dependent zone of inhibitions obtained. The highest diameter of zone of inhibition (14.23 mm) was obtained at 100 mg/mL by n-hexane extract against *E. coli* and *S. aureus*, respectively, while the lowest zone of inhibition (6.00 mm) was obtained by n-hexane at 25 mg/mL concentrated tested against *S. aureus.* However, the diameter of the zone of inhibition of Ciprofloxacin (50 mg/mL) ranged between 10-16 mm for E. coli and 6-10 mm for S. aureus. At the same time, 14.00 mm was obtained by itraconazole (antifungal agent) against *C. albicans*. Thus, this study shows that *E. lateriflora* harbors active compounds and supports the claim that its extract may be effective against infections caused by the test organisms, as practiced for years among rural dwellers in Nigeria.

Keywords: *Euphorbia lateriflora*, phytochemical screening, HPLC, disc diffusion assay, caffeic acid.

1. Introduction

Resistance among bacteria is a threat to the effectiveness of antibiotics and limits the therapeutic alternatives for most infections [1]. This justifies the resurgence of interest in the investigation and development of potential antibacterial agents from natural materials as a source of drug for the treatment of infections [2]. In West Africa, particularly Nigeria, medicinal plants have shown distinguishing features in herbal therapy due to their popularity,

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potent activities, and generally very little to no toxicity. Scientists believe that medicinal plants may reduce the pressure on synthetic substances [3].

Biologically active compounds obtained from pure sources can be therapeutic on the health of humans [4]. Countless contemporary drugs used in therapy are developed from pure sources [5]. Thus, one technique used to discover antimicrobial agents is through the evaluation of plants for medicinal potentials [6].

Euphorbia lateriflora belonging to the largest family (Euphorbiaceae) of plants [7] is known among Nigerians [8] as "*Enu Opiri"* (Yoruba) and "*Fidda sartse*" (Hausa). Traditionally, the leaves have been used to remedy many ailments including dermatitis [9]. Significantly too, numerous scientific reports have documented the potential of the leaves against an array of microorganisms and to treat many diseases, including chickenpox [10, 11]. However, Ganga *et al*. [12] reported a scarcity of information on the pharmacological potential of plants in the genus Euphorbia. Thus, this study investigated the secondary metabolites and *in vitro* efficacies of the n-hexane and ethyl acetate extracts of *E. lateriflora* against selected pathogens.

2. Materials and Methods

Leaves of *E. lateriflora* were obtained from Islamic Village, Ilorin, Nigeria. The leaf was authenticated at the Herbarium Unit of the Department of Plant Biology, the University of Ilorin where a voucher specimen was deposited UILH/003/2020/1268. Following the method described by Delahaye-Mckenzie *et al*. [13], the leaves were thoroughly rinsed with distilled water in the laboratory and reduced into smaller sizes. It was further air-dried for 11 days and pulverized into powder using a clean Blender (Master Chef Blender, Model MC-BL 1980, China).

The powdered leaves were weighed and kept in an air-tight container and stored at 4^{o}C until further use. Serial dilution of the extract was done to generate decreasing concentrations. Sterility testing of the dilutions was done by transferring 2 mL of the solution (extract) into 10 mL of respective medium (broth) for each test organism, this was incubated at 37 ^{o}C and 28 ^{o}C for 24 and 72 hours, respectively, after which they were checked for clarity as an indication of sterility. This was repeated to check for sterility of ethyl acetate extract.

2.1 Preparation of Crude Extract

The extraction was done by setting up two sterile conical flasks containing 1000 mL of the solvents (n-hexane and ethyl acetate). Each flask was labeled A and B, and 200 g of the powdered *E. lateriflora powder* was dispensed into each flask (1:5 w/v). The conical flasks were covered with cotton wool, wrapped with aluminium foil, and allowed to stay for 48 hours with intermittent shaking to facilitate extraction [14, 15].

The filtrates recovered from the percolates were then completely evaporated under pressure using the rotary evaporator (Model RE Zhengzhou, Henan China). The weight of the concentrated extracts was determined before storage in sterile and labeled air-tight containers. They were kept in the refrigerator at 4^{o}C until needed for analysis. Each extract was constituted by dissolving in 5% dimethylsulphoxide (DMSO) to have varying concentrations of 25, 50, and 100 mg/mL.

2.2 Test Microbial strains

The microbial strains "*E. coli*, *S. aureus,* and *C. albicans*" used in the present study were collected on Nutrient, and Sabouraud dextrose agar (Oxoid) slants, respectively. All tested microorganisms were sub-cultured to check for purity and later pre-cultured on nutrient and PDA broth overnight and for 72 hours at 37 ^{o}C and 28^{o}C, respectively. The test isolates (0.1mL) were inoculated into their respective broths and incubated for 18 and 48 hours, respectively, at 37 °C/ 28^{o}C. Further dilution of the microbial suspensions was done with normal saline (0.85 %), and the turbidity was adjusted to McFarland number by comparing with standard tube (containing 1.5×10^{8} CFU / mL and 10^{6} Conidia/mL).

2.3 Preliminary Phytochemical screening

The aqueous extracts of *E. lateriflora* were screened to detect bioactive components such as alkaloid, saponin, Glycoside, Tannin, flavonoid, steroid, terpenoid, phenolics, coumarins, triterpenes, phlobatannins, amino acids, and Anthocyanins using standard procedures as described below [16].

2.3.1 Alkaloid Test "Wagner's test"

Into 0.l ml of each extract (ethyl acetate and n-hexane), three drops of Wagner's reagent "Iodine in Potassium iodide" were added, and the formation of a brown precipitate, which indicated the presence of alkaloids was observed.

2.3.2 Glycosides Test "Liebermann's test"

Into 2 ml of chloroform, two ml of each extract (ethyl acetate and n-hexane) and acetic acid were added. The solution was cooled in ice, and then sulphuric acid was added carefully. The presence of a steroidal nucleus was reported as a color change from violet to bluish-green.

2.3.3 Saponins Test "Foam Test"

Five ml of each extract (ethyl acetate and n-hexane) were added to 5 ml of distilled water. This was shaken vigorously and warmed. The resulting formation of stable foam indicated the presence of saponins.

2.3.4 Tannin Test "Gelatin test"

Into 2ml of each extract (ethyl acetate and n-hexane),1% solution containing sodium chloride and gelatin was added. The formation of white precipitate indicated the presence of tannins.

2.3.5 Terpenoid Test "Liebermann-Burchard test"

Into a test tube containing 2ml of chloroform and 3ml of concentrated tetraoxosulphate (vi) acid was added 5ml of each extract (ethyl acetate and n-hexane). The presence of terpenoids was indicated by forming a reddish-brown color at the interface.

2.3.6 Steroids Test "Liebermann-Burchard test"

To 0.2 g of each portion of extract (ethyl acetate and n-hexane), 2 ml of acetic acid was added; the solution was cooled well in ice followed by the addition of conc. H_2SO_4 carefully. Color development from violet to blue or bluish-green indicated the presence of a steroidal ring i.e., aglycone portion of cardiac glycoside

2.4 Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

The serial broth dilution technique was employed to ascertain the MIC/MFC of the plant extracts against the organisms using the method described by Altuner *et al*. [17]. Different extract concentrations of 100, 50, 25, 12.5 and 6.25 mg/mL were set up in sterile test tubes. A quantity of 1.0 mL of 100 mg/mL of the extract solution was successfully transferred into 1ml of nutrient broth to obtain extract concentrations as set up.

Subsequently, 1 ml of an 18 hours broth culture which had previously been modified to 0.5 McFarland equivalence of 1.0 x 10^{8} CFU/m was introduced into each test tube. The test tubes were further vortexed, and incubation was done at 37 ^{o}C for 24 hours and at 28 ^{o}C for 72 hours for the yeast isolate. The MIC was taken as the tube with no detectable growth, and this tube indicated 99.5% killing of the original inoculum.

2.5 Evaluation of the antimicrobial activity of the E. lateriflora extract

The antimicrobial effect of the solvent "n-hexane and ethyl acetate" extracts of *E. lateriflora* was evaluated against the selected pathogens using the modified disc diffusion assay described by the Kirby Bauer technique [18, 19]. Twenty mL (20 mL) of molten (cooled to 45°C) Mueller Hinton agar (MHA) was allowed to solidify. The same media was supplemented with 2% glucose and methylene blue for *C. albicans*, after which, 0.2 mL of an overnight broth culture of the standardized test organism was streaked on MHA to make a complete lawn.

Sterile Whatman. No. 1 filter paper discs (6 mm) soaked with the extract were plated on the inoculated MHA plates. There was also control setups: a negative control (5% DMSO), positive control (10 µg/disk "Oxoid Ltd" of ciprofloxacin against the bacterial strains and itraconazole against *C. albicans*). All plates were incubated for 24 hours at 37 ^{o}C and 72 hours at 28 ^{o}C for the yeast. The diameters of the zone of inhibitions (clear zones around the discs) were measured using a measuring scale and recorded in millimeter (mm). The experiment was done in triplicate for each solvent extract.

2.6 HPLC Analysis

The HPLC technique was employed to identify compounds in the extract. This technique was conducted using a SHMADZU SCL-10 AVP C18 column "100*4.6mm, 5µm particle size". The column was equipped with an auto-sampler alongside a UV detector with the mobile phase (containing 1.0% acetic acid) as methanol– acetonitrile-water "40:15:45 v/v/v". The column was eluted "at 1.0 ml per minute while the chromatographic peak of the data was confirmed by comparing their retention time and UV spectra with the reference standard.

3. Results

Phytochemical screening of *E. lateriflora* extracts revealed the presence of varying compounds in the solvent "n-hexane and ethyl acetate" extracts (Table 1)

Table 1- Phytochemical constituents of the extracts of ***E. lateriflora***

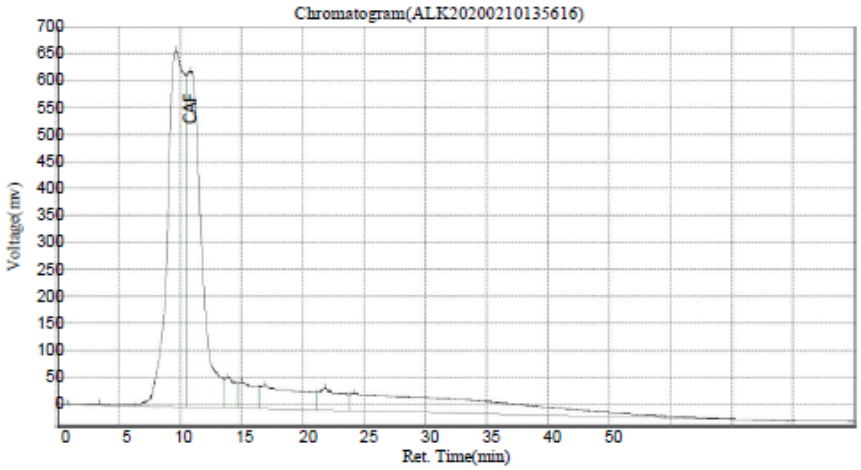
Parameters	n-Hexane	Ethyl acetate
Saponins	-	+
Tannins	-	-
Phenolics	-	-
Flavonoids	+	+
Coumarins	+	+
Anthocyanins	-	-
Steroids	+	+
Glycosides	+	+
Triterpenes	+	+
Terpenoids	+	+
Phlobatannins	-	-
Amino acids	-	-
Alkaloids	+	+

In Figure 1, the HPLC chromatogram of Ethyl acetate extract of *E. lateriflora* detected nine compounds, one of which was identified as caffeic acid which was detected at 10 and 14 min.

Instrument:LC
Column Temp.(¡æ)£º40

Gradient:

Detector:DAD
Wavelength(nm)£º205

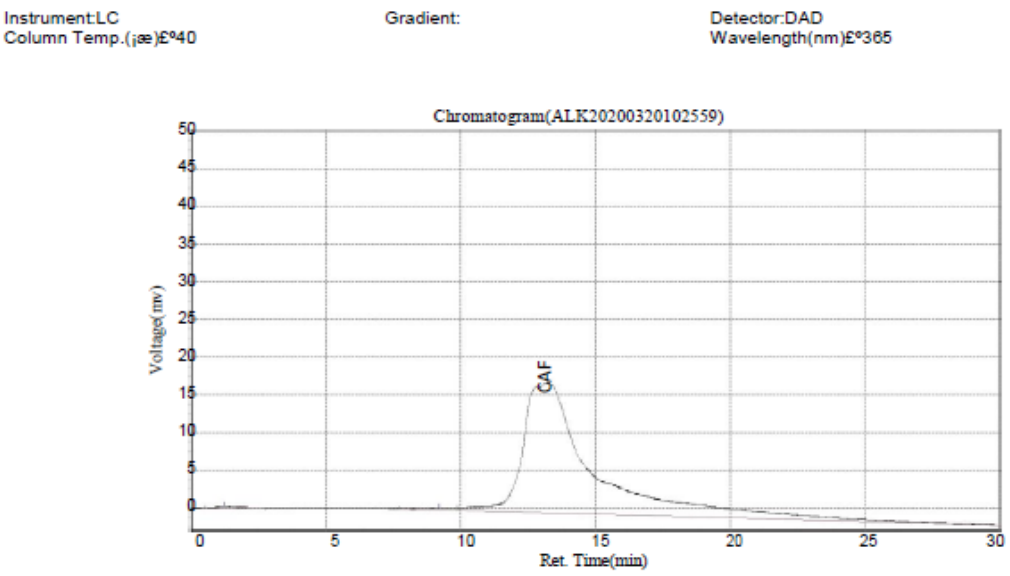


Results

Peak No.	Peak ID	Ret Time	Height	Area	Conc mg/100g
1	Unidentified	0.148	597.082	3718.611	0.0109
2	Unidentified	8.665	2883.087	154948.219	0.4552
3	Caffeic Acid	10.923	660761.063	13162393.000	108.6642
4	Caffeic Acid	14.165	623143.750	10205885.000	59.9795
5	Unidentified	15.773	56598.543	667103.938	1.9596
6	Unidentified	17.007	48562.383	915509.938	2.6893
7	Unidentified	22.365	44332.531	2043152.500	6.0017
8	Unidentified	24.365	40574.992	1056236.875	3.1027
9	Unidentified	32.840	31531.563	5833884.500	1.1369

Figure 1- HPLC chromatogram of Ethyl acetate extract of *E. lateriflora*

However, in Figure 2, the HPLC chromatogram of n-Hexane extract of *E. lateriflora* detected three compounds. The highest compound detected at 13 minutes was identified as caffeic acid.



Results

Peak No.	Peak ID	Ret Time	Height	Area	Conc mg/100ml
1	Unidentified	2.240	252.189	2592.850	0.4084
2	Unidentified	8.840	262.088	4381.748	0.6903
3	Caffeic Acid	13.640	17123.658	627828.125	14.9013

Figure 2-HPLC chromatogram of n-Hexane extract of *E. lateriflora*

The MIC of ethyl acetate (Table 2) extract of *E. lateriflora* showed that various concentrations of the extract could prevent the growth of the tested isolates. MIC range of 6.25, 12.5 and 25 mg/mL was established by the extract against all the tested isolates, while MIC/MFC value of 50 mg/mL was only shown against *E. coli* and *C. albicans*, and that of 100mg /mL was shown against *C. albicans.*

Table 2- MIC and MFC of Ethyl acetate Extract

Dilutions of ethyl acetate extract of *E. lateriflora* (mg/mL)					
Test strains	100	50	25	12.5	6.25
E. coli	-	+	+	+	+
S. aureus	-	-	+	+	+
C. albicans	+	+	+	+	+

The MIC of n-hexane (Table 3) extract of *E. lateriflora* showed that dilutions of various concentrations of the extracts could inhibit the growth of the tested isolates. The extracts showed MIC range of 6.25, 12.5 and 25 mg/mL against all the tested isolates while no MIC value was observed for 50 mg/mL and 100mg /mL.

Table 3- MIC of n-hexane Extract

	Dilutions of n-hexane extract of *E. lateriflora* (mg/mL)				
Test strains	100	50	25	12.5	6.25
E. coli	-	-	+	+	+
S. aureus	-	-	+	+	+
C. albicans	-	-	+	+	+

The range of zone of inhibition by extracts of *E. lateriflora* was noted to be from 6.00-14.23mm. The highest diameter of the zone of inhibition (14.23 ±1.19 mm) was obtained at 100 mg/mL by n-hexane extract against *E. coli* and *S. aureus*. While the lowest zone of inhibition (6.00mm) was obtained by n-hexane at 25mg/mL concentrated tested against *S. aureus.*

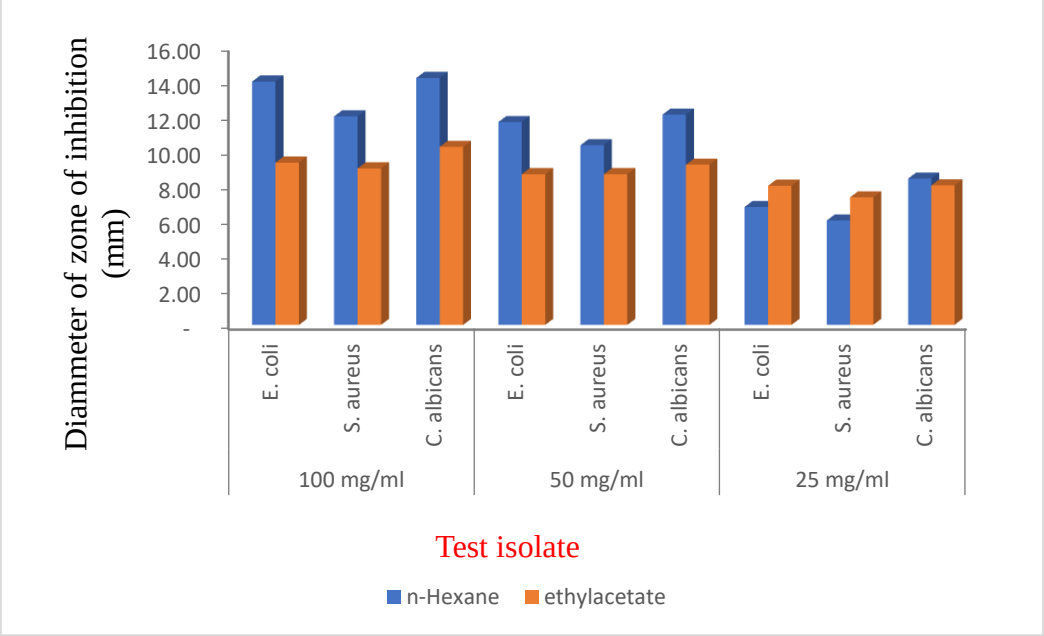


Figure 3-Diameter of zones of inhibition of different concentrations of solvent "n-Hexane and ethyl acetate" extracts of *E. lateriflora* against the test isolates

The zones of inhibition presented in Figure 4 show that the control agents' activities vary on the isolates tested. The zone of inhibition of the commercial agents "Ciprofloxacin at 50 mg/mL" ranged between 10-16 mm on *E. coli* and 6-10 mm on *S. aureus*, while 14.00 ±1.08 mm was obtained by itraconazole at 50 mg/mL against *C. albicans*.

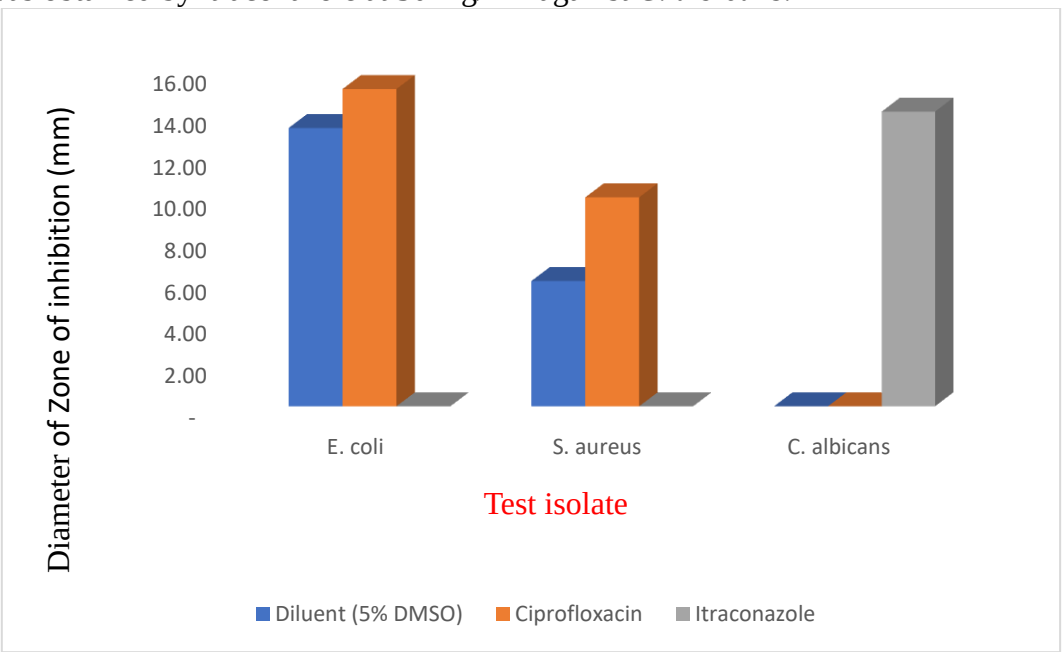


Figure 4-Diameter of zones of inhibition of the control agents against the test isolates

4. Discussion

Plants and their natural products have been used worldwide, including Nigeria, as either extracts or infusions in the management of diseases [20]. Approximately 163 species of medicinal plants were used in the preparations of agents for wound healing in Ayurvedic systems of medicine [21, 22]. Consequently, this study investigated the efficacy of *Euphorbia lateriflora* against three common pathogens associated with wound infections, namely *Staphylococcus aureus, Escherichia coli, and C. albicans*. According to Pfaller and Diekema [23], Candida spp. has been the fourth leading cause of clinically acquired infections It has been reported to be associated with infections of different parts of the body [24]. Due to the cell wall peptidoglycan components, *Staphylococcus aureus* is associated with wound infections [25, 26], while *E. coli* is reported to be the causal agent of necrotizing fasciitis [27], surgical site infections [28], and other wound-related infections

In this study, the preliminary phytochemical screening of extracts of *E. lateriflora* revealed varying important phytochemical components "alkaloid, tannin, saponin, glycoside, steroid and alkaloids, flavonoid, terpenoid, coumarins". A similar report has been given by Seigler [29] on the presence of tannins, alkaloids, diterpenes, triterpenes in Euphorbia spp. However, this study conversely reported the absence of tannin in both solvent extracts of *E. lateriflora* screened. Since antiquity, the pharmacological properties of plants have been linked to the presence of various secondary compounds such as tannins, saponins, glycosides, flavonoids, steroids, alkaloids and terpenes which are important in combating many disease-causing pathogens [30, 31]. Doughari and Okafor [32] also mentioned that some compounds (such as alkaloids, saponins, and tannins) had been reported to possess inhibitory potentials against bacteria.

Previous reports on the screening of phytochemical compounds of *E. lateriflora* extracts showed the presence of various compounds "tannins, lectins, diterpenes, euphorbin, alkaloids ingenol mebutate, anhydriobisfarnesol, and sesquilavandulol" [33]. Contrarily, this study identified Caffeic acid along with numerous unidentified compounds in the HPLC analysis of the solvent extracts of *E. lateriflora*. This may mean that other solvents should be employed, and a stronger stationary phase should be used. Identification of a polyphenol, i.e. Caffeic acid, may also account for the antimicrobial properties of this plant. Polyphenols have shown a promising antimicrobial potential against *S. aureus*, and with low toxicity towards human cells [34]. These findings may suggest the antimicrobial potentials of this plant, as supported by the report of Doughari and Okafor [32]. They also stated that the bioactivity of medicinal plants on humans had been attributed to their phytochemical composition.

It is a well-established challenge that drug-resistance pathogens have a high prevalence [35]. Hence, a significant concern is shown by researching alternative therapies that could help alleviate problems associated with microbial drug resistance leading to delayed wound healing [36]. Interestingly, Euphorbia species have been reportedly used to manage wounds in ethnomedicine [37]. This study suggests that the in vitro antimicrobial activities of E. lateriflora further support its wound healing potential. According to Govindarajan *et al*. [38], the foremost measure towards determining antimicrobial efficacy of plants is by the *in vitro* antibacterial activity assay. This study thus revealed a lower MIC by n-hexane extract than ethyl acetate extract; this was further demonstrated by higher antimicrobial activities of n-hexane extract suggesting its better bactericidal potentials than ethyl acetate.

Independent of the concentration tested, the n-hexane and ethyl acetate extracts of *E. lateriflora* showed varying appreciable potentials against the tested pathogens. This result agrees with Sudhakar *et al.* [39], who equally reported a wide spectrum of antimicrobial activity by Euphorbia species against varieties of organisms including *Staphylococcus aureus*. The result also suggested that the solvent extracts displayed some degrees of activity even at the lowest tested concentration. However, n-hexane extract showed better activity against *E.*

coli at the highest concentration tested. While the antimicrobial activity exhibited by the positive control was higher than what was achieved by either of the solvent extracts against *E. coli and C. albicans*, less activity was recorded against *S. aureus*, the activity of 5% DMSO was higher than that of ethyl acetate against the test pathogens. Using the appropriate solvent is one of the significant factors influencing antimicrobial activity because of the difficulty in resolubilizing extracts. The choice of solvent used for extraction impacts the activity of the extract [40]. DMSO is a stable solvent with high polarity and can serve as an exceptional solvent property [41] for resolubilizing a wide range of plant extracts. The antimicrobial activity of DMSO and other solvents have been reported in some scientific literatures [42]. Also, better activity exhibited by the synthetic drugs may be due to their better solubility as reported by Cowan [43] that most of the antibiotic compounds are aromatic or saturated organic molecules that can easily solubilize.

The present study concluded that *E. lateriflora* has antimicrobial potential and this can be attributed to the secondary metabolites present in it. However, further study is needed to determine the purity of the unknown compounds. Also, there is a need to identify more active components by using different solvents to extract the leaves of *E. lateriflora.*

5. Conclusion

It is concluded from the results of this work that extracts of *E. lateriflora* possess active constituents with good antimicrobial activity against the tested pathogen. So, the claim of its use against infections associated with the test pathogens is corroborated. Hence, this plant could be a reliable source for developing potential antimicrobial drugs. However, further study is warranted to screen the components of this plant that may be of value in the pharmacological industry.

6. Acknowledgments

7. Conflict of interest

The author declares no conflict of interest.

8. References

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