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ORIGINAL ARTICLE

Comparative Analysis of the Antimicrobial Potential of Stem and Fruit Extracts of *Calotropis procera*

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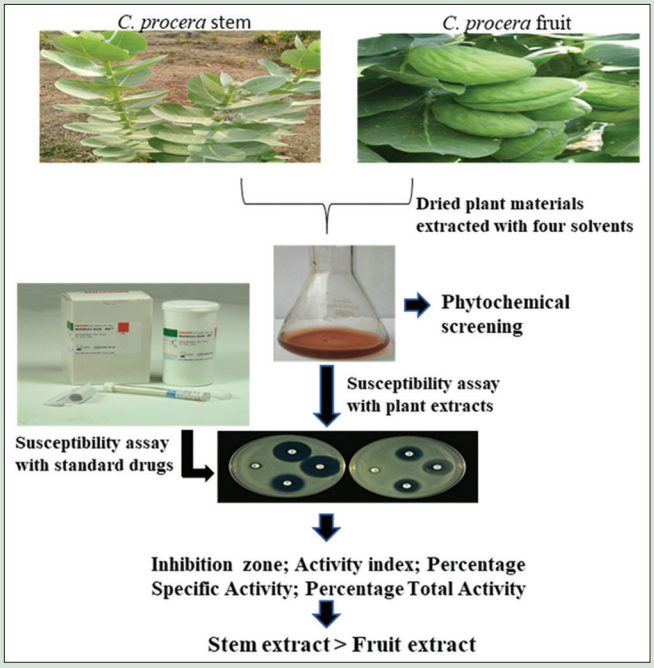
ABSTRACT

Background: The dramatic rise in antimicrobial resistance continues to threaten the effective management and treatment of emerging and re-emerging infectious diseases. Medicinal plants offer excellent therapeutic alternative especially due to their plethora bioactive constituents and low resistance development to them. **Purpose:** The comparative analysis of the antimicrobial potential of the stem and fruit extracts of *Calotropis procera* was investigated. **Materials and Methods:** Using different extracts of the plant, the phytochemical screening was determined alongside their antimicrobial properties with agar-disc diffusion assay. The antimicrobial potency of the plant extracts (200 mg/ml) was evaluated and compared by their inhibition zone (IZ), activity index (AI), percentage specific activity (PSA), and percentage total activity (PTA) values. **Results:** The highest antibacterial activity (IZ = 15 ± 0.5 mm) was displayed by the stem hot aqueous extract against *Staphylococcus aureus*, while the maximum antifungal effect was exerted by both the stem cold and hot aqueous extracts ($P > 0.05$). The overall antimicrobial AI (AI = 1.56) was displayed by the stem cold aqueous extract against *Candida albicans*. The stem's hot and ethanolic extracts exhibited the highest achievable PSA of 100%. Furthermore, the stem extracts displayed the PTA of 83.3% compared to 25% by the fruit extracts, thus confirming the greater antimicrobial potency of the plant's stem extracts. **Conclusion:** This study suggests that while the stem extracts of *C. procera* could have displayed better antimicrobial activity, the overall effects elicited by the plant could be attributed to the presence of phytochemicals as revealed by the result of the phytochemical screening. Further studies focusing on complete characterization and evaluation of the mechanism of antimicrobial action of bioactive constituents of the extracts is underway.

Key words: Antimicrobial activity, antimicrobial resistance, *Calotropis procera*, medicinal plants, phytochemicals

SUMMARY

- The antimicrobial activity of the stem and fruit extracts of *Calotropis procera* against *Staphylococcus aureus*, *Escherichia coli,* and *Candida albicans* was compared. The plant extracts contain phytochemicals such as saponins, tannins, flavonoids, alkaloids, phenols, steroids, phytosterols, and terpenoids. The antimicrobial potential of the stem extracts was found to be greater that of the fruit extract, thus suggesting the better efficacy of the stem extracts. Further studies focusing on complete characterization and evaluation of the mechanism of antimicrobial action of bioactive constituents of the extracts is underway.



Abbreviations Used: CSC: *Calotropis procera* stem cold aqueous extract; CSH: *Calotropis procera* stem hot aqueous extract; CSE: *Calotropis procera* stem ethanolic extract; CSM: *Calotropis procera* stem methanolic extract; CFC: *Calotropis procera* fruit cold aqueous extract; CFH: *Calotropis procera* fruit hot aqueous extract; CFE: *Calotropis procera* fruit ethanolic extract; CFM: *Calotropis procera* fruit methanolic extract; IZ: inhibition zone; AI: activity index; PSA: percentage specific activity; PTA: Percentage total activity.

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INTRODUCTION

The landmark discovery of the first medical miracle, penicillin, by Sir Alexander Fleming in 1928 marked the modern era of antibiotics.[1,2] Since then, antibiotics have been used to improve the quality of lives, save lives, and reduce economic burden posed by infectious diseases.[3,4] However, the inappropriate use and overuse of antibiotics in human and veterinary medicine have led to dramatically increased resistance in both bacteria and fungi.[5] Antimicrobial resistance (AMR) is an

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eminent public health crisis responsible for reduced quality of lives, increased hospitalization, and increased economic burden. The impacts of AMR in developing nations like Nigeria are even more amplified due to inadequate medical resources.[6,7] Furthermore, it has been estimated that, by 2050, some 10 million annual mortality will be attributable to AMR, if no practical alternatives are sought.[8] In pursuit of novel and alternative treatment options, natural sources such as medicinal plants are being explored and could serve as possible sources of novel and affordable antimicrobial agents.[9,10]

Plants have been harnessed as a medicinal source since primordial times. The traditional and folk medicines utilize plant products for the treatment of various infectious diseases.[11] In addition, plants have also been shown to possess anticancer, anti-inflammatory, immunomodulatory, antimicrobial, and antioxidant properties.[12-14] These properties elicited by plants are due to their plethora of bioactive constituents such as alkaloids, flavonoids, tannins, saponins, terpenoids, sugars, amino acids, proteins, et cetera.[15,16] Furthermore, several studies have lent credence to the potential use of plant materials for novel drug discovery and development.[17-19]

Calotropis procera (Ait.) R. Br., with common names, Sodom apple, swallowwort, dead sea apple, milkweed (English), *kisher* (Arabic), *pomme de sodome* (French), *bomubomu* (Yoruba), and *tumfafiya* (Hausa), is a wild-growing plant that belongs to the family Asclepiadaceae. It is endemic to and widely distributed in the tropical and subtropical regions of Africa and Asia.[20,21] Traditionally, *C. procera* is used to treat ailments such as fever, rheumatism, pain, asthma, bronchitis, ulcer, indigestion, cold, eczema, measles, diarrhea, abscesses, and jaundice.[21,22] More specifically, its stem is used for the treatment of elephantiasis, cough, asthma, dysentery, and skin diseases (e.g., eczema and leprosy).[23] The leaf possesses analgesic and antinociceptive properties and is also used in snakebite antidote.[24] Table 1 highlights few pharmacological properties of the plant.

Although studies have reported the antimicrobial properties of various parts of *C. procera*, none provided a comprehensive comparison of the antimicrobial property of the stem and fruit extracts of the plant.[22,24,33-35] The present study attempted to provide a comprehensive report on the comparative analysis of the antimicrobial potential of the stem and fruit extracts of *C. procera*.

MATERIALS AND METHODS

Test micro-organisms

Already identified and characterized clinical isolates, including *Staphylococcus aureus*, *Escherichia coli,* and *Candida albicans,* were obtained from the Microbiology Laboratory, Al-Hikmah University, Ilorin. The isolates were maintained on agar slants at 4°C for future use.

Collection and preparation of plant materials

Fresh and healthy stems and fruits of *C. procera* [Figure 1] were collected from a garden near Al-Hikmah University (8.482" N, 4.505" E), Ilorin, Nigeria. The plant materials were washed, air-dried, and pulverized into a fine homogeneous powder using a sterile ceramic mortar and pestle. The powdered material was weighed and kept in an air-tight container before extraction.

Table 1: Few pharmacological potentials of *Calotropis procera* plant

Pharmacological property	Plant part used	Preparation	Phytochemical constituent	References
Antidiarrheal potential	Dry latex	Castor oil-induced diarrhea model was used. The plant dry latex delayed the onset of diarrhea and a significant number of rats were afforded protection against the induced diarrhea	-	[25]
	Leaf	70% hydroethanolic extract of *C. procera* reduced fecal boluses and improved diarrhea severity in a castor oil-induced diarrhea model	-	[26]
Anti-inflammatory activity	Latex	The plant latex extracts had better activity than standard drug (phenylbutazone) at suppressing/ reducing injuries induced by carrageenin	-	[27]
Antimicrobial property	Leaf and Latex	The ethanolic latex extract showed great potency against tested microorganisms	Alkaloids, flavonoids, saponins, and tannins	[21]
	Leaf	The crude flavonoid fraction displayed remarkable antibacterial (15.5-28.5 mm) and anti-candidal (30 mm) activity	Flavonoids, which include quercetin-3-O-rutinoside, kaempferol-3-O-rutinoside, isorhamnetin-3-O-rutinoside	[28]
Antitumor effect	Root	The plant extract arrested the HepG2 cell at S phase, prevented initiation of G2/M phase and apoptosis induction	-	[29]
	Latex	Treatment of mice with dried latex of plant afforded treated animals' absolute protection against hepatocarcinogenesis. No contraindication was also observed	-	[30]
Anthelmintic activity	Flower	Although with lower activity than standard drug (levamisole), the plant flower extracts showed good *in vitro* and *in vivo* anthelmintic activity against nematodes	-	[31]
Wound healing property	Latex	Excision was made on the back of guinea pigs. This was followed by topical application of *C. procera*. The latex-treated animal showed increased collagen fibers, DNA, and increased protein synthesis resulting in the healing of the wound area	-	[32]

C. procera: Calotropis procera

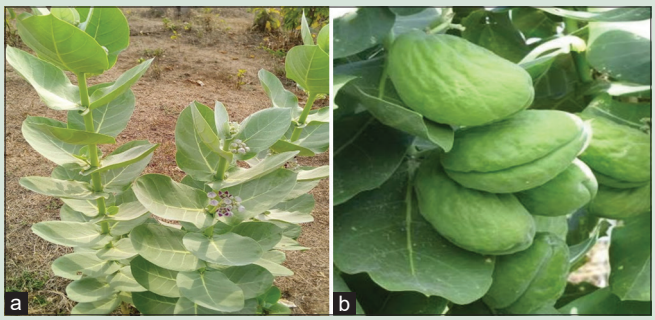

Figure 1: *Calotropis procera* plant showing its long and slender stem (a) and fruit (b)

Preparation of plant extracts

The plant extraction was done as earlier described with slight modifications.[36] Briefly, 70 g powdered plant materials were prepared separately in 700 ml of low-to-high polarity solvents, namely ethanol, methanol, and distilled water (cold and hot); the plant extraction was done using a rotary shaker for 48 h. The resulting extract was filtered with a sterile filter paper (Whatman No. 1), and the filtrate was evaporated and dried using a water bath at 40°C for 48 h. The concentrated extract was subsequently scraped into a sterile bottle with a sterile spatula and stored at 4°C for future use.

Qualitative phytochemical screening

The phytochemical (saponins, tannins, flavonoids, alkaloids, phenols, steroids, and phytosterols) constituents of all different plant extracts which include *C. procera* stem cold aqueous extract (CSC), *C. procera* stem hot aqueous extract (CSH), *C. procera* stem ethanolic extract (CSE), *C. procera* stem methanolic extract (CSM), *C. procera* fruit cold aqueous extract (CFC), *C. procera* fruit hot aqueous extract (CFH), *C. procera* fruit ethanolic extract (CFE), and *C. procera* fruit methanolic extract (CFM) were qualitatively determined as described below.

Test for saponins

This was done by frothing test with few modifications of previous method.[37] Briefly, 50 mg of each plant extract was dissolved in distilled water (5 ml) and filtered. The resulting filtrate was homogenized vigorously and warmed (10 min). The formation of a stable foam confirmed the presence of saponins.

Detection of tannins

Briefly, 50 mg of each extract was mixed with distilled water (2 ml). This was filtered and accompanied by the addition of few drops of 10% $FeCl_3$ solution to the filtrate. A brownish-green or blue-black coloration/precipitation when compared with the control indicates the presence of tannins.[37,38]

Detection of flavonoids

Briefly, 25 mg of each extract was dissolved in 2 ml diluted NaOH. This was filtered and followed by the addition of few drops of hydrochloric acid (HCl) into the filtrate. A yellow coloration when compared with the control compared indicated the presence of flavonoids.[39]

Detection of alkaloids

Briefly, 25 mg of each extract was dissolved with 3 ml of 1% HCl; the mixture was heated and then filtered. Mayer's and Wagner's reagents were added to the resulting filtrate. A yellowish precipitate confirmed the presence of alkaloids.[37]

Detection of phenols

Briefly, 50 mg of each extract was dissolved in distilled water (2 ml), filtered, and accompanied by the addition of ferric chloride solution (2 ml) to the filtrate. The formation of blue-black or brown color confirmed the presence of phenols.[40]

Detection of phytosteroids

Briefly, 50 mg of each extract was dissolved in 2 ml chloroform and filtered, and two drops of concentrated sulfuric acid were added to the filtrate. This was homogenized and left for some minutes. The presence of a golden yellow color when compared with the control confirmed the presence of phytosteroids.[39]

Detection of steroids

For this, Liebermann–Burchard's test was performed. Briefly, 50 mg of each extract was dissolved in 5 ml chloroform and filtered; 2 ml sulfuric acid and 2 ml acetic anhydride were serially added to the resulting filtrate. A color change from violet to blue-green when compared with the control sample confirmed the presence of steroids.[37,41]

Test for terpenoids

Briefly, 50 mg of each extract was dissolved in 2 ml chloroform and filtered, and 2 ml sulfuric acid was subsequently added to solution. Formation of reddish-violet color when compared with the control was an indication of terpenoids' presence.[42]

Standard antibiotic and antifungal susceptibility assay

This was done to determine the susceptibility profiles of the tested isolates and was carried out using the agar-disc diffusion method. Briefly, overnight nutrient broth cultures of each bacterial strain were standardized to a turbidity of 0.5 McFarland standard, and standardized yeast culture (OD_{600} = 0.5) was also prepared. Dried surface of Mueller-Hinton agar (MHA) or Sabouraud's dextrose agar (SDA) plate was swabbed with standardized test isolate's inoculum (100 µl). Commercially purchased antibiotics discs or antifungal-loaded discs were placed onto the agar surface, and the plates were incubated at 37°C (24 h) and 37°C (24–48 h) for the bacterial and fungal strains, respectively. The inhibition zone (IZ) was measured in millimeter and was compared with CLSI antibiogram standards.[10,43,44]

Evaluation of the antimicrobial activity of plant extracts

The antimicrobial activity of the *C. procera* stem and fruit's extracts was evaluated using the agar-disc diffusion method. Briefly, 200 mg/ml concentration of each extract was prepared by dissolving 0.4 g of respective extract in distilled water (2 ml). Subsequently, overnight nutrient broth cultures of each bacterial strain were standardized to a turbidity of 0.5 McFarland standard. The standardized culture (100 µl) of each bacterial strain was evenly spread on MHA surface using a sterile hockey stick. Standard discs (6 mm in diameter) impregnated with plant extract (200 mg/ml) were equidistantly placed onto the agar and incubated at 37°C for 24 h. For the yeast strain, 100 µl of an overnight Sabouraud's dextrose broth (SDB) culture (OD_{600} = 0.5) of *C. albicans* was evenly spread on SDA surface. Subsequently, the plant extract-loaded discs (200 mg/ml) were placed onto the agar and incubated at 37°C for 24–48 h. Discs of gentamicin (10 µg/ml)

and ketoconazole (25 µg/ml) were used as standard drugs (positive controls) for the antibacterial and antifungal assays, respectively. Negative controls were set up with discs impregnated with sterile distilled water. The antimicrobial activity was recorded as the diameter of IZ formed around the discs.[10,43,44]

Other antimicrobial activity indexes

The activity index (AI) of each plant extract relative to the drug standard was calculated to express the relationship between the extract and the reference drug.[45,46]

$$AI = \left(\frac{\text{Inhibition zone of each extract}}{\text{Inhibition zone of reference drug}} \right)$$

The percentage specific activity (PSA) to determine the antimicrobial activity of each extract against all tested isolates was calculated.

$$PSA = 100\left(\frac{\text{Number of susceptible isolates to a specific extract}}{\text{Total number of isolates treated with the extract}} \right)$$

While the percentage total activity (PTA) of the extracts of each plant material (i.e., stem or fruit) was also evaluated, this was important to determine the overall antimicrobial activity of each plant material. As an example, the PTA for the stem extracts was calculated as:

$$PTA = 100\left(\frac{\text{Number of times stem extracts was active}}{\text{Total number of times stem extracts was tested}} \right)$$

Statistical analysis

The data obtained with respect to the zones of inhibition (mean ± standard deviation) of the extracts were compared with that of standard drugs using one-way analysis of variance complemented with Tukey's multiple comparisons test (www.graphpad.com). A significant difference was taken at $P < 0.05$ and indicated on the graph by different letters.

RESULTS

Qualitative phytochemical screening

The results of phytochemical screening of *C. procera* stem and fruit extracts are presented in Table 2. The presence of saponins, tannins, flavonoids, alkaloids, phenols, steroids, phytosterols, and terpenoids was observed in at least one of the plant extracts. However, tannins and steroids were absent in all the stem extracts, while the fruit extracts were all devoid of alkaloids [Table 2]. Moreover, saponins, phenols, and flavonoids appear to be the most abundant secondary metabolites in the plant materials evident by their presence in at least six out of the eight extracts screened.

Table 2: Qualitative phytochemical screening of plant materials

Test	Stem extracts				Fruit extracts			
	CSC	CSH	CSE	CSM	CFC	CFH	CFE	CFM
Saponins	+	+	-	+	+	+	+	+
Tannins	-	-	-	-	+	-	-	+
Flavonoids	+	+	-	+	+	+	+	-
Alkaloids	-	+	-	-	-	-	-	-
Phenols	+	+	-	+	+	+	+	+
Steroids	-	-	-	-	+	-	-	-
Phytosterols	-	-	+	-	-	-	+	+
Terpenoids	ND	ND	ND	ND	+	+	+	+

+: Detected; -: Not detected; ND: Not determined; CSC: *C. procera* stem cold aqueous extract; CSH: *C. procera* stem hot aqueous extract; CSE: *C. procera* stem ethanolic extract; CSM: *C. procera* stem methanolic extract; CFC: *C. procera* fruit cold aqueous extract; CFH: *C. procera* fruit hot aqueous extract; CFE: *C. procera* fruit ethanolic extract; CFM: *C. procera* fruit methanolic extract; *C. procera*: *Calotropis procera*

Standard antibiotic and antifungal susceptibility assay

The antimicrobial susceptibility profiles of the tested isolates against standard antibiotics and antifungal drugs are shown in Table 3. The *S. aureus* strain used in this study was resistant to six out of the eight standard antibiotics tested, thus indicating its multidrug resistance phenotype. However, the *E. coli* strain used was only resistant to ceftazidime and augmentin. In addition, the yeast strain used (*C. albicans*) in this study was susceptible to nystatin and ketoconazole but was resistant to the most commonly used antifungal drug, fluconazole.

Evaluation of the antimicrobial activity of plant extracts

The antimicrobial potential of the plant extracts was determined using the agar-disc diffusion method. The three microbial strains tested were sensitive to at least one of the plant extracts, excluding the CFC and CFE. The highest activity (IZ = 15 ± 0.5 mm) against *S. aureus* was recorded for CSH, and this compared favorably with that exerted by gentamicin ($P > 0.05$) [Figure 2a]. However, CSC, CFC, CFH, CFE, and CFM had no antibacterial effects against *S. aureus* ($P > 0.05$). For *E. coli*, CSC exhibited the highest antimicrobial activity (IZ = 9 ± 0.50 mm) among all extracts screened [Figure 2b]. This was followed by CSH, CSE, and CFH ($P > 0.05$), however, the standard drug (gentamicin) had the overall highest activity (IZ = 17 ± 0.0 mm) against *E. coli* ($P < 0.05$) [Figure 2b]. Moreover, the tested *E. coli* strain was markedly resistant to CSM, CFC, CFE, and CFM ($P > 0.05$).

As shown in Figure 3, among all the eight extracts and one standard drug (ketoconazole) screened against the yeast strain, CSC and CSH displayed the

Table 3: Antimicrobial susceptibility profiles of tested isolates with standard drugs

Test strain	Inhibition zone (mm)													
	CAZ	CRX	CXC	ERY	CTR	OFL	GEN	AUG	CXM	NIT	CPR	NYS	KET	FLU
S. aureus	12±1.0 (R)	6±0.0 (R)	6±0.5 (R)	6±1.0 (R)	23±0.0 (R)	26±3.0 (S)	17±1.5 (S)	6±0.5 (R)	ND	ND	ND	ND	ND	ND
E. coli	18±2.5 (I)	7±1.0 (R)	ND	ND	ND	25±1.0 (S)	17±0.0 (S)	9±0.5 (R)	17±1.5 (I)	21±0.5 (S)	20±1.5 (I)	ND	ND	ND
C. albicans	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	24±2.0 (S)	16±0.5 (R)	0±0.0 (R)

CAZ (30µg): Ceftazidime; CRX (30µg): Cefuroxime; CXC (5µg): Cloxacillin; ERY (5µg): Erythromycin; CTR (30µg): Ceftriaxone; OFL (5µg): Ofloxacin; GEN (10µg): Gentamicin; AUG (30µg): Augmentin; CXM (5µg): Cefixime; NIT (300µg): Nitrofurantoin; CPR (5µg): Ciprofloxacin; NYS (25µg/ml): Nystatin; KET (25µg/ml): Ketoconazole; FLU (25µg/ml): Fluconazole; ND: Not determined; *S. aureus: Staphylococcus aureus; E. coli: Escherichia coli; C. albicans: Candida albicans*

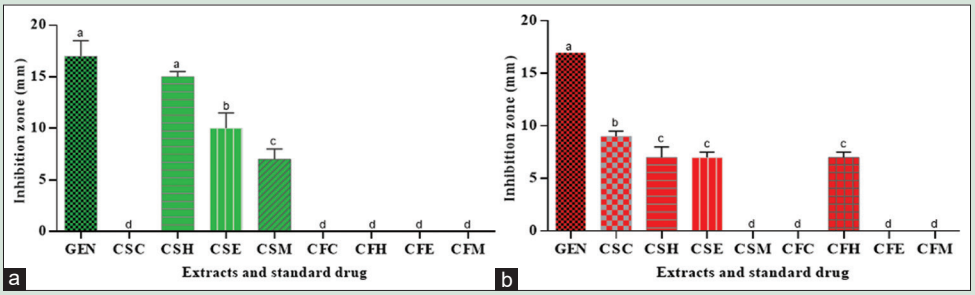

Figure 2: Antibacterial activity of *Calotropis procera* stem and fruit extracts against (a) *Staphylococcus aureus* and (b) *Escherichia coli* bars with different letters being significantly different ($P < 0.05$)

most pronounced antifungal activities (IZ = 25 ± 0.5 mm and IZ = 23 ± 2.0 mm, respectively) ($P > 0.05$). The antifungal effect (IZ = 16 ± 0.5 mm) of the standard drug was favorably comparable to that exerted by CSE, CSM, and CFM ($P > 0.05$) but slightly higher than what was displayed by CFH (IZ = 10 ± 0.5 mm) ($P < 0.05$). However, the yeast strain was resistant to CFC and CFE ($P > 0.05$).

Antimicrobial susceptibility indexes such as AI, PSA, and PTA were used to compare the antimicrobial effects of the stem and fruit extracts of *C. procera* against the tested isolates [Table 4]. The highest antibacterial AI was recorded for CSH (AI = 0.88) against *S. aureus*. This was followed by AI of 0.59 and 0.41 for CSE and CSM, respectively, against the same organism. The maximum antifungal AI against the yeast was exhibited by CSC (AI = 1.56), followed by CSH (AI = 1.44) and CSE (AI = 1.06).

The highest PSA of 100% was recorded for CSH and CSE, indicating that the two extracts were active against all tested isolates. This was followed by PSA of 66.7% for CSC, CSM, and CFH, respectively. However, no activity was displayed by CFC and CFE against all the tested isolates. Finally, the comparison of the PTA of the stem and fruit extracts, as shown in Table 4, indicates that the stem extracts exhibited the overall highest PTA of 83.3%, while that of the fruit extracts was 25.0%, thus indicating the better activity of the stem extracts.

Table 4: Antimicrobial activities of *Calotropis procera* stem and fruit extracts against all tested microbial strains

Plant material	Extract	Antimicrobial activity index				
		S. aureus	*E. coli*	*C. albicans*	All isolates	
		AI^g	AI^g	AI^k	PSA (%)	PTA (%)
Stem	CSC	0.00	0.36	1.56	66.7	83.3
	CSH	0.88	0.28	1.44	100.0	
	CSE	0.59	0.28	1.06	100.0	
	CSM	0.41	0.00	0.94	66.7	
Fruit	CFC	0.00	0.00	0.00	0.0	25.0
	CFH	0.00	0.28	0.63	66.7	
	CFE	0.00	0.00	0.00	0	
	CFM	0.00	0.00	0.88	33.3	

AI^g: Activity index relative to gentamicin; AI^g: Activity index relative to ketoconazole; PSA: Percentage specific activity; PTA: Percentage total activity; CSC: *C. procera* stem cold aqueous extract; CSH: *C. procera* stem hot aqueous extract; CSE: *C. procera* stem ethanolic extract; CSM: *C. procera* stem methanolic extract; CFC: *C. procera* fruit cold aqueous extract; CFH: *C. procera* fruit hot aqueous extract; CFE: *C. procera* fruit ethanolic extract; CFM: *C. procera* fruit methanolic extract; *C. procera*: *Calotropis procera*; *S. aureus*: *Staphylococcus aureus*; *E. coli*: *Escherichia coli*; *C. albicans*: *Candida albicans*

DISCUSSION

The dramatic rise in AMR development continues to threaten the effective management and treatment of emerging and re-emerging infectious diseases, and this consequently increases the morbidity, mortality, and economic burdens associated with such infections. Medicinal plants represent an alternative, inexpensive, and rich source of antimicrobial agents due to the profusion of bioactive compounds they contain.[15,16] In the present study, the antimicrobial activity of the stem and fruit extracts of *C. procera* against *S. aureus*, *E. coli,* and *C. albicans* was assayed, while the antimicrobial potentials of the two plant parts were also compared. Aside from the dramatic rise and increased threat displayed by AMR, the clinical isolates used during this study were selected because of their clinical significance; *S. aureus* is the leading etiology of both nosocomial and community-acquired infections and largely implicated in septicemia, pneumonia, and wound infections;[47] *E. coli* is a prominent cause of enteritis and urinary tract infection,[48] while *C. albicans* remains the predominant cause of invasive fungal infections.[49]

The qualitative phytochemical screening of *C. procera* stem and fruit extracts revealed the presence of saponins, tannins, flavonoids, alkaloids, phenols, steroids, phytosterols, and terpenoids in at least one of the plant extracts [Table 2]. The presence of these secondary metabolites in the plant extracts may explain their antimicrobial effects since secondary metabolites are known to possess antimicrobial, anticancer, antimalarial, and antioxidant properties.[12-15] Furthermore, the presence of saponins in the methanolic extracts of the fruit and stem of *C. procera* agrees with previous findings.[11,50] On the other hand, the absence of alkaloids in the methanolic extracts of both fruit and stem also coheres with findings of previous study.[11] Moreover, the presence of flavonoids in the aqueous extracts of the plant stem is consistent with the report of a previous study.[33]

The antimicrobial susceptibility patterns of the tested isolates with standard drugs revealed that the *S. aureus* used in this study is probably a multidrug-resistant (MDR) strain due to its resistance to six out of the eight tested antibiotics. The MDR phenotype displayed by this strain may be due to the synthesis of β-lactamase which causes hydrolysis of β-lactam ring, thus resulting in inactivation of β-lactam antibiotics.[51] On the other hand, the *E. coli* strain was only resistant to two out of the eight tested antibiotics. Moreover, the yeast strain was resistant to fluconazole, which is the most commonly used antifungal drug due to its low toxicity, great efficacy, high bioavailability, and high-water solubility.[52]

In general, all the plant extracts screened displayed enormous antimicrobial property, with only two out of the eight extracts screened exhibiting no antibacterial or antifungal property. The highest antibacterial activity (IZ = 15 ± 0.5 mm) was displayed by CSH against *S. aureus*, while the maximum antifungal effect was exerted by CSC and CSH ($P > 0.05$).

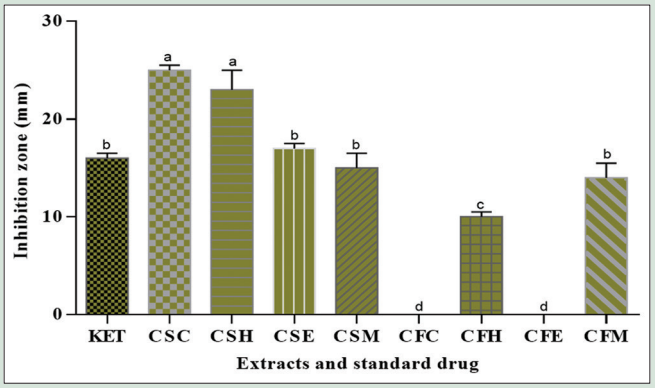

Figure 3: Antifungal effect of *Calotropis procera* stem and fruit extracts on *Candida albicans*

The antimicrobial potential of the plant extracts was also assessed by evaluating AI of each extract relative to standard drug. Gentamicin was used as the reference drug for the bacterial isolates, while ketoconazole was used for the yeast strain. For this, the best antibacterial AI was exhibited by CSH (AI = 0.88) against *S. aureus*, while the maximum antifungal (AI = 1.56) against the yeast was displayed by CSH. This indicates that the overall antimicrobial (AI = 1.56) was displayed by CSH. The highest achievable PSA of 100% was exhibited by CSH and CSE; this suggests the broad-spectrum activities of these two extracts against all tested isolates. However, the CFC and CFE had a specific activity of 0% which translates to their non-antimicrobial effects against all the tested strains. Furthermore, the stem extracts displayed the PTA of 83.3% compared to 25% exhibited by the fruit extracts, thus suggesting the better efficacy and greater antimicrobial potency of the plant's stem extracts.

CONCLUSION

Our present study reveals and confirms the plethora of phytochemical constituents of *C. procera* stem and fruit's extracts. It also suggests the better potency and efficacy of the stem extracts compared with the fruit extracts of the plant. These findings validate and expand our current knowledge of the antimicrobial potentials of medicinal plants, plant products, and secondary metabolites. However, further studies should focus on complete characterization and evaluation of the mechanism of antimicrobial action of bioactive constituents of the extracts.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Piddock LJ. The crisis of no new antibiotics – What is the way forward? Lancet Infect Dis 2012;12:249-53.
2. Sengupta S, Chattopadhyay MK, Grossart HP. The multifaceted roles of antibiotics and antibiotic resistance in nature. Front Microbiol 2013;4:47.
3. Gould IM, Bal AM. New antibiotic agents in the pipeline and how they can help overcome microbial resistance. Virulence 2013;4:185-91.
4. CDC. Antibiotic Resistance Threatens Everyone [Internet]. Centers for Disease Control and Prevention. 2020. Available from: http://www.cdc.gov/drugresistance/. Last Accessed on 29th April 2020.
5. Saadabi MA. Evaluation of *Lawsonia inermis* Linn. (Sudanese Henna) leaf extracts as an antimicrobial agent. Res J Biol Sci 2007;2:419-23.
6. Marasini BP, Baral P, Aryal P, Ghimire KR, Neupane S, Dahal N, *et al*. Evaluation of antibacterial activity of some traditionally used medicinal plants against human pathogenic bacteria. Biomed Res Int 2015;2015:265425.
7. Jamiu AT, Bello SA. Biosynthesis of silver nanoparticles using azadirachta indica leaf extract and assessment of its antibacterial activity on some pathogenic enteric bacteria. Int J Nov Res Life Sci 2018;5:25-31.
8. O'Neill J. Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations [Internet]. 2014. Available from: http://www.jpiamr.eu/wp-content/uploads/2014/12/AMR-Review-Paper-Tackling-a-crisis-for-the-health-and-wealth-of-nations_1-2.pdf. 2nd June 2020.
9. Kitula RA. Use of medicinal plants for human health in Udzungwa Mountains Forests: A case study of New Dabaga Ulongambi Forest Reserve, Tanzania. J Ethnobiol Ethnomed 2007;3:7.
10. Bello SA, Jamiu AT. Antibacterial activity of *Moringa oleifera* seed extracts on *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Niger J Microbiol 2017;31:3873-81.
11. Morsy N, Al Sherif EA, Abdel-rassol TM. Phytochemical analysis of *Calotropis procera* with antimicrobial activity investigation. Main Group Chem 2016;15:267-73.
12. Nanasombat S, Teckchuen N. Antimicrobial, antioxidant and anticancer activities of Thai local vegetables. J Med Plants Res 2009;3:443-9.
13. Veeru P, Kishor M, Meenakshi M. Screening of medicinal plant extracts for antioxidant activity. J Med Plants Res 2009;3:608-12.
14. Sudha P, Asdaq SM, Dhamingi SS, Chandrakala GK. Immunomodulatory activity of methanolic leaf extract of *Moringa oleifera* in animals. Indian J Physiol Pharmacol 2010;54:133-40.
15. Krishnaiah D, Devi T, Bono A, Sarbatly A. Studies on phytochemical constituents of six Malaysian medicinal plants. J Med Plants Res 2009;3:67-72.
16. Tshabalala BD, Alayande KA, Sabiu S, Ashafa AO. Antimicrobial and anthelmintic potential of root and leaf extracts of *Gazania krebsiana* Less. subsp. serrulata (DC.) Roessler: An *in vitro* assessment. Eur J Integr Med 2016;8:533-9.
17. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev 1999;12:564-82.
18. Cragg GM, Newman DJ. Biodiversity: A continuing source of novel drug leads. Pure Appl Chem 2005;77:7-24.
19. Bakal SN, Bereswill S, Heimesaat MM. Finding novel antibiotic substances from medicinal plants – Antimicrobial properties of *Nigella sativa* directed against multidrug-resistant bacteria. Eur J Microbiol Immunol (Bp) 2017;7:92-8.
20. Sehgal R, Roy S, Kumar VL. Evaluation of cytotoxic potential of latex of *Calotropis procera* and podophyllotoxin in *Allium cepa* root model. Biocell 2006;30:9-13.
21. Shobowale OO, Ogbulie NJ, Itoandon EE, Oresegun MO, Olatope SO. Phytochemical and antimicrobial evaluation of aqueous and organic extracts of *Calotropis procera* ait leaf and latex. Niger Food J 2013;31:77-82.
22. Kareem S, Akpan I, Ojo O. Antimicrobial activities of *Calotropis procera* on selected pathogenic micro-organisms. Afr J Biomed Res 2008;11:105-10.
23. Abhishek D, Mohit C, Ashish G, Ameeta A. Medicinal utility of *Calotropis procera* (Ait.) R. Br. as used by natives of village Sanwer of Indore District, Madhya Pradesh. Int J Pharm Life Sci 2010;1:188-90.
24. Farouk A-EA, Ahamed NT, AlZahrani O, Alamer KH, Bahobail AA. Antimicrobial activities evaluation from the extracts of leaves, flowers, fruits and latex of *Calotropis procera* from Taif. Int J Curr Microbiol Appl Sci 2016;5:240-56.
25. Kumar S, Dewan S, Sangraula H, Kumar VL. Anti-diarrhoeal activity of the latex of *Calotropis procera*. J Ethnopharmacol 2001;76:115-8.
26. Murti Y, Singh A, Pathak D. Comparison of antidiarrheal activity of hydroethanolic extracts of *Calotropis procera* and *Calotropis gigantea* leaves. J Pharm Sci Innov 2012;1:32-3.
27. Arya S, Kumar VL. Antiinflammatory efficacy of extracts of latex of *Calotropis procera* against different mediators of inflammation. Mediators Inflamm 2005;2005:228-32.
28. Nenaah G. Antimicrobial activity of *Calotropis procera* Ait. (Asclepiadaceae) and isolation of four flavonoid glycosides as the active constituents. World J Microbiol Biotechnol 2013;29:1255-62.
29. Mathur R, Gupta SK, Mathur SR, Velpandian T. Anti-tumor studies with extracts of *Calotropis procera* (Ait.) R.Br. root employing Hep2 cells and their possible mechanism of action. Indian J Exp Biol 2009;47:343-8.

30. Choedon T, Mathan G, Arya S, Kumar VL, Kumar V. Anticancer and cytotoxic properties of the latex of *Calotropis procera* in a transgenic mouse model of hepatocellular carcinoma. World J Gastroenterol 2006;12:2517-22.
31. Iqbal Z, Lateef M, Jabbar A, Muhammad G, Khan MN. Anthelmintic activity of *Calotropis procera* (Ait.) Ait. F. flowers in sheep. J Ethnopharmacol 2005;102:256-61.
32. Rasik AM, Raghubir R, Gupta A, Shukla A, Dubey MP, Srivastava S, *et al*. Healing potential of *Calotropis procera* on dermal wounds in Guinea pigs. J Ethnopharmacol 1999;68:261-6.
33. Mainasara MM, Aliero BL, Aliero AA, Dahiru SS. Phytochemical and antibacterial properties of *Calotropis procera* (Ait) R. Br. (Sodom Apple) fruit and bark extracts. Int J Mod Bot 2011;1:8-11.
34. Bashyal S, Rai S, Abdul Manan O, Hashmi F, Guha A. Preliminary phytochemical testing and antimicrobial activity of *Calotropis procera* leaves. Int J Trend Sci Res Dev 2017;2:926-30.
35. Asoso OS, Oladunmoye MK. Ogundare. Antibacterial screening of crude fractions of *Calotropis procera* (Linn) and Gc-Ms profile of fractions obtained from the extracts of *Calotropis procera*. Biomed J Sci Tech Res 2019;18:13418-424. DOI: 10.26717/BJSTR.2019.18.003124.
36. Varghese S, Narmadha R, Gomathi D, Kalaiselvi M, Devaki K. Phytochemical screening and HPTLC finger printing analysis of *Citrullus lanatus* (Thunb.) seed. J Acute Dis 2013;2:122-6.
37. Vaghasiya Y, Dave R, Chanda S. Phytochemical analysis of some medicinal plants from Western region of India. Res J Med Plant 2011;5:567-76.
38. Odebiyi OO, Sofowora EA. Phytochemical screening of Nigerian medicinal plants II. Lloydia 1978;41:234-46.
39. Okwu DE, Ekeke O. Phytochemical screening and mineral composition of chewing sticks in South Eastern Nigeria. Glob J Pure Appl Sci 2003;9:235-8.
40. Benavente-García O, Castillo J. Update on uses and properties of citrus flavonoids: New findings in anticancer, cardiovascular, and anti-inflammatory activity. J Agric Food Chem 2008;56:6185-205.
41. Auwal MS, Tijjani AN, Sadiq MA, Saka S, Mairiga IA, Shuaibu A, *et al*. Antibacterial and haematological activity of *Moringa oleifera* aqueous seed extract in Wistar albino rats. Sokoto J Veterinary Sci 2013;11:28-37.
42. Ezeonu CS, Ejikeme CM. Qualitative and quantitative determination of phytochemical contents of indigenous Nigerian softwoods. New J Sci 2016;2016:1-9.
43. National Committee for Clinical Laboratory Standards. Performance Standards for Antimicrobial Susceptibility Testing 8th Information Supplement. M100S12. Villanova, Pa. Ilanova, Pa: National Committee for Clinical Laboratory Standards; 2011.
44. Al-Enazi NM, Awaad AS, Zain ME, Alqasoumi SI. Antimicrobial, antioxidant and anticancer activities of *Laurencia catarinensis*, *Laurencia majuscula* and *Padina pavonica* extracts. Saudi Pharm J 2018;26:44-52.
45. Singariya P, Mourya K, Kumar P. Antibacterial and antifungal potential of some polar solvent extracts of Ashwagandha (Solanaceae) against the nosocomial pathogens. Int J Green Pharm 2012;6:17.
46. Rajput M, Kumar N. *In vitro* Antimicrobial and antibiofilm efficacy of medicinal plant extracts against clinical MDR isolates from scalp infection cases. Int J Sci Technol Res 2020;9:4218-28.
47. Ansari S, Nepal HP, Gautam R, Rayamajhi N, Shrestha S, Upadhyay G, *et al*. Threat of drug resistant *Staphylococcus aureus* to health in Nepal. BMC Infect Dis 2014;14:157.
48. Allocati N, Masulli M, Alexeyev MF, Di Ilio C. *Escherichia coli* in Europe: An overview. Int J Environ Res Public Health 2013;10:6235-54.
49. Sardi JC, Scorzoni L, Bernardi T, Fusco-Almeida AM, Mendes Giannini MJ. Candida species: Current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. J Med Microbiol 2013;62:10-24.
50. Shrivastava A, Singh S, Singh S. Phytochemical investigation of different plant parts of *Calotropis procera*. Int J Sci Res Publ 2013;3:1-4.
51. Fuda C, Hesek D, Lee M, Morio K, Nowak T, Mobashery S. Activation for catalysis of penicillin-binding protein 2a from methicillin-resistant *Staphylococcus aureus* by bacterial cell wall. J Am Chem Soc 2005;127:2056-7.
52. Andriole VT. Current and future antifungal therapy: New targets for antifungal therapy. Int J Antimicrob Agents 2000;16:317-21.