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SUSCEPTIBILITY OF MICROBIAL INFECTIOUS AGENTS TO THE LATEX AND LEAF EXTRACT OF *Calotropis procera*

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ABSTRACT

The age-old traditional usage of *the Calotropis procera* plant for the treatment of several infectious diseases has established the plant as ethnomedicinal with widely reported exploitation in current herbal medicinal practice. This prompted the investigation of the in vitro susceptibility of various disease-causing microorganisms to various doses of the latex and leaf extract of *the C. procera* plant. The latex and leaves of the *C. procera* plant were collected and processed before subjecting the leaf to ethanol extraction via the maceration protocol. The various concentrations of the latex and leaf extract of *C. procera* were subjected to antimicrobial susceptibility tests using the agar well diffusion technique. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined according to standard microbiological methods. The *C. procera* latex exhibited antimicrobial activity against microbial isolates even at the lowest concentration of 20 % (v/v) except for *E. coli* which was not susceptible at this concentration. Lower antimicrobial activity was observed with the leaf extract of *C. procera* even though antifungal activity against *C. albican* was high with both the latex and the leaf extract. The MIC of the *C. procera* latex detected at 12.5 and 25 % (v/v) and MBC at quarter strength of the latex also emphasized the inhibitory and bactericidal capability of the *C. procera* latex against bacterial infectious agents of clinical origin. The study attributed the antimicrobial potency of the *C. procera* latex and leaf extract to its phytochemicals as widely referred in the literature. As per findings from this study, *C. procera* could serve as a valuable pharmacological prospect for the development of new natural antimicrobial drugs from plant sources that could be useful in combating today's menace of antibiotic resistance.

Keywords: *Calotropis procera,* Antimicrobial Activity, Leaf and Latex Extract, Minimum Inhibitory Concentration, Minimum Bactericidal Concentration.

INTRODUCTION

Diseases caused by microbial infectious agents have become the main cause of mortality, additionally, and the resistance of microorganisms to antimicrobial drugs has increasingly threatened global public health (Biharee *et al.,* 2020). Today, the research world and pharmaceutical outfits are in a rigorous race for the discovery of new potent antimicrobial drugs for these health challenges. The therapeutic use of medicinal plants dates back to the ancient periods and researchers now refer to the historical medicinal benefits of plants to search for new alternative antimicrobial drugs from plant sources. Consequently, the recognized potency of medicinal plants based on their vast pharmacological functions has enabled contemporary exploitation; and now, medicinal plants and herbs remain the foremost source of primary health care in emerging countries (Mulat *et al.,* 2020).

Calotropis procera is among the widely reported ethno medicinal plant supposedly

used in traditional settings for the treatment of several illnesses including infectious ones (Pathania *et al.,* 2020). Mohammad *et al.,* (2021) reported relevant literature about the traditional medicinal application of *C. procera* including plant parts and medication from different countries. *C. procera* is a common plant from the family Apocynaceae, a xerophyte, tall, highly branched perennial shrub having stems of 2 to 6 m tall with tap roots of 3 to 4 m deep in the soil (Hassan *et al.,* 2015; Mali *et al.,* 2019). The plant is characterised by a thick milky sap also known as latex which exudes from the plant when its parts are cut (Waikar & Srivastava, 2015). The *C. procera* plant also known as Sodom apple is distributed in various tropical and subtropical regions and found growing in dry sand and alkaline soils, mostly in waste land growing abundantly as a weed. (Hassan *et al.,* 2015; Mainasara *et al.,* 2012) The leaf of *C. procera* is large ovate, sessile, opposite, and up to 30 cm in length and 16 cm in width (Aliyu, 2006).

The *C. procera,* commonly known as Giant Indian milked weed (Manduzai *et al.,* 2021), is a well-known medicinal plant of Ayurveda, Siddha, Arabic, Sudanese and Unani local and traditional medicines (Pathania *et al.,* 2020), and famous to the Greeco-Arab medicine since the ancient period and used by olden days Egyptians as medicines during the Neolithic period (Hassan *et al.,* 2015). Among the early traditional medicinal use of the *C. procera* plant is for the treatment of a various array of ill health conditions such as fever, jaundice, tumours, body pains and various infectious diseases and many more (Mascolo *et al*., 1988; Tounekti *et al*., 2019). Vohra, (2004) reported that the root when dried is used as therapy for asthma, bronchitis, elephantiasis, leprosy, hepatic and splenic enlargement and eczema while the dried whole plant is consumed as a medicinal tonic, expectorant and an antihelmintic (Warrier *et al.,* 1996). Mainasara *et al.,* (2012) also stated that sap or latex has been used to treat boils, wound infections, human skin problems and for treating skin infestation by parasites in animals. Also, the topical application of the sap for cutaneous treatment of diseases such as leprosy, ringworm, and syphilitic sores (Kew, 1985), psoriasis, scorpion, eczema, and snake bites have been reported (Mohammad *et al.,* 2021). The *C. procera* plants portrayed ethnobotanical uses with assorted biological value for the treatment of diseases globally before the advent of modern clinical drugs. To accentuate these therapeutic claims, recently, much research has been focused towards the establishment of empirical evidence to support the use of plants, especially in medicinal practices (Ojo *et al.,* 2005). Therefore, this study aimed at investigating the susceptibility of infectious disease agents to the leaf extract and latex of the *C. procera* plant to determine its antimicrobial and pharmacological value.

MATERIALS AND METHODS

Collection of Leaf and Latex of *C. procera* of Plant

The *Calotropis procera* plant found behind the Faculty of Science building (Gombe State University, Gombe) was initially identified using the standard keys and descriptions as per Keay, (1989). The suspected *C. procera* plant was then taken to the herbarium in the Plant Biology Department for botanical and taxonomical identification and authentication plant by a botanist. After the confirmation of the plant identity, the leaves of *C. procera* were plugged and then collected for further processing. For the collection of the latex otherwise known as sap, the leaves were initially wiped clean before extracting or collecting the latex from the leaves into a sterile container under aseptic condition to

avoid contamination then stored in the refrigerator at 4 °C before use.

Preparation and Processing of the Leaves of *C. procera*

The leaves of *the C. procera* plant collected were wiped clean using sterile cotton wool to eliminate dirt and then rinsed with distilled water. The leave devoid of water were air-dried at room temperature for 2 weeks as reported by Ibrahim *et al.,* (2015). The dried leaves of *C. procera* were processed by grinding them into a powder using a blender and then the powdery products obtained were stored in a dry and airtight container.

Production of Leaf Extract of *C. procera* via Ethanol Extraction

The extraction method reported by Parekh and Chanda (2007) was used for the ethanolic extraction. 50 grams of the powdered *Calotropis procera* leaves were macerated in 500 mL of ethanol in a conical flask for 14 days at room temperature with constant shaking on the rotary shaker. The soaked coloured mixture obtained was filtered with muslin cloth and then Whatman's filter paper. The filtrate collected was heat-evaporated to obtain an extract concentrate, afterwards, the crude leaf extract obtained was preserved in the refrigerator at 4 °C.

Sterility Test on the Ethanolic Leaf Extract and Latex of *C. procera*

To verify the sterility status of the ethanolic leaf extract and latex of *C. procera* for the susceptibility test, the sterility test protocol described by Okolo *et al.,* (2019) was adopted. This was achieved by adding 2 mL of the ethanolic leaf extract (re-constituted with distilled water) and latex into separate tubes containing 10 mL of Muller-Hinton broth and then incubating at 37 °C for 24 hours. The detection of clearness of the broth after incubation which implies the absence of turbidity indicates a sterile extract, on the contrary, the presence of turbidity in the tubes (as a result of microbial growth) is indicative of contaminated or non-sterile extract.

Collection of Test Microorganisms of Clinical Origin

Test organisms such as *Staphylococcus aureus, Salmonella typhi, Escherichia coli and Candida albican* from clinical samples were collected from the microbiology laboratory of the Federal Teaching Hospital Gombe state.

Sub-culturing and Purification of Test Organisms

Basic microbiology techniques such as sub-culturing were initially carried out on Nutrient agar and then incubated at 37 °C for 18 to 24 hours to obtain fresh isolates. To obtain a pure culture, colonies from the overnight plates were streak-plated on another nutrient agar and then incubated at 37 °C for 24 hours for discrete pure colonies. *Candida albican* was purified via the same streak-plating method on Sabouraud Dextrose Agar (SDA) (with added Chloramphenicol) and then incubated at 25 °C for 2 to 5 days.

Microbial Isolate Identification and Confirmation

Using standard microbiological techniques such as Gram staining and microscopy, biochemical tests, and Germ tube fermentation, the purified or pure culture of the microbial test organisms were further identified and confirmed as elaborated below:

Gram Staining technique and Microscopy

To identify the Gram reaction of the bacterial test organisms, Gram staining was carried out on the purified culture of test organisms based on the methodology described by Cheesbrough (2005). Afterwards, the Gram-stained cells were examined under the microscope with x100

objective lens to observe the cell's microscopic morphology.

Biochemical Analyses for Bacterial Confirmation

Further confirmation of the test bacterial isolates was achieved by adopting the various biochemical tests namely, catalase, coagulase, indole, citrate, motility, and urease tests. The established procedures for biochemical tests by Cheesbrough, (2002) were applied.

Germ Tube Test for Confirmation of Candida albican

The pure culture of *Candida albican* was subjected to a Germ tube fermentation test for the confirmation of the fungal isolate. As reported by Cheesbrough, (2006), 0.5 mL of human serum was dispensed in a test tube and then inoculated with *C. albican* using a sterile wire loop. The inoculated tube was incubated at 35 to 37 °C for 2 to 3 hours in a water bath and then a drop of the incubated mixture was transferred onto a glass slide and covered with a cover slip. Sprouting yeast cells mostly tube-like outgrowths was examined from the slide under the microscope at x10 and x40 objective lens.

Preparation of Stock and Standard Concentration of Leaf extract and Latex of *C. procera*

A stock concentration of 100 mg/mL of the *C. procera* leaf extract was prepared as described by Ibrahim *et al.,* (2015), by dissolving 100 mg of the leaf extract in 1 mL of distilled water. Lower standard concentrations of 70, 50 and 30 mg/mL of the leaf extract were further prepared in separate sterile bijou bottles by appropriate dilution with distilled water from the stock. For the preparation of standards for the latex of *C. procera*, the method reported by Ibrahim *et al.*, (2020) was adopted where the undiluted sap or latex collected was used as 100 % (v/v). From the undiluted stock, lower standards of 80, 60, 40 and 20 % (v/v) were prepared.

Antimicrobial Sensitivity Test

The in vitro antimicrobial susceptibility test was achieved using the agar well or ditch diffusion method as per the National Committee for Clinical Laboratory Standards NCCLS (1993) to determine the antimicrobial activity of the leaf extract and latex of *C. Procera* against the various test microbes. 20 to 25 mL of Mueller-Hinton agar were aseptically poured into empty Petri dishes and then allowed to set. A sterile swab stick was inserted inside the standardized inoculum and then inoculated by evenly swabbing the complete surface of the Mueller-Hinton agar plate. Using a sterile 6 mm cork borer, five (5) agar wells were made on the surface of the inoculated Mueller-Hinton agar then 0.2 mL of the stock and standard concentrations (100, 80, 60, 40 and 20 % (v/v)). For the leaf extract, 0.2 mL of the prepared concentrations (100, 70, 50 and 30 mg/mL) were dispensed in separate wells. For both plates, the Gentamycin antibiotic disc (10µg) was carefully placed at the centre as a positive control. All the plates were allowed for some minutes for proper diffusion of the extracts into the agar wells and then the plates were incubated in an inverted position for 24 hours at 37 °C. After incubation, the diameter zone of inhibition (DZI) produced around the wells were measured in millimetre using a meter rule. For the anti-fungal susceptibility test, Sabouraud Dextrose Agar (SDA) containing Chloramphenicol was used to prevent any bacterial contamination. Plates for the anti-fungal activity test were incubated at 25 °C for 72 hours. Antimicrobial susceptibility assay was done in replicates.

Minimum Inhibitory Concentration (MIC) Determination

The MIC assay was carried out with only the latex of *C. procera* using the tube dilution method (TDM) or dilution broth method reported by Gutpe, (2006). Initially, the undiluted stock (100 % (v/v)) was used to prepare half concentrations via double dilution with distilled water until five lower standard concentrations of 50, 25, 12.5, 6.2 and 3.1 % (v/v) were obtained in separate bijou bottles. 1 mL from the new standard concentrations (50, 25, 12.5, 6.3 and 3.1 % (v/v)) of the *C. procera* latex was dispensed into five different sterile test tubes, then 1 mL of Muller-Hinton broth was added to all five tubes which yielded a final concentration of approximately 25, 12.5, 6.3, 3.1 and 1.5 % (v/v) for the MIC bioassay. 50 µL of the standardized inoculum was introduced into the test tubes, gently vortexed then incubated at 37 °C for 24 hours. Five control tubes were set up containing only standards of the latex and the Mueller-Hinton broth as previously described without inoculation then incubated all together. All the tubes were examined for microbial growth after 24-hour incubation by checking for turbidity and comparing them with their respective control tubes (with no growth). Consequently, the tubes that showed no growth signified that the test organism was inhibited by the latex of *C. procera.* The lowest concentration of the latex of *C. procera* that inhibited the growth of the test organisms as determined by no turbidity in the tubes was recorded as the minimum inhibitory concentration (MIC).

Minimum Bactericidal Concentration (MBC) Determination

This was performed to determine the least concentration of the *C. procera* latex that could kill the test microorganisms. The MBC method described by Nwankwo and Chika, (2017) was applied where the negative tubes that show no bacterial growth during the MIC determination were selected, and then a loopful from each of the negative tubes was spread-plated on Mueller-Hinton agar using a sterile swab stick. The inoculated plates were incubated at 37 °C for 24 hours and then MBC was determined as the least concentration of the leaf or stem bark extract that showed no colony or visible growth on the Mueller-Hinton agar plate.

RESULTS

Table 1 shows the qualitative result of the sterility test on the crude extract of the leaf and the latex of *C. procera.*

Table 1: Sterility Test on the Leaf Extract and Latex of *C. procera*

Crude extracts	Turbidity detection
Leaf extract	-
Latex or sap	-

Table 1 shows the sterility status of the latex and leaf extract of C. procera where the negative sign (-) implies no detection or absence of turbidity.

The crude leaf extract and latex of *C. procera* showed no turbidity in the tubes after 24-hour incubation which implies no microbial growth. Accordingly, the leaf extract and latex of *C. procera* used for the assessment of antimicrobial activity were sterile (Table 1).

In Table 2, the susceptibility of the test bacterial isolates to the different concentrations of *C. procera* latex is shown by the production of diameter zone of inhibition (DZI). For the bacterial isolates, 7 mm was the lowest DZI produced by *S. typhi* at the lowest concentration of 20 % (v/v) while *E. coli* produced the same DZI at 40 % (v/v) with no activity at the lowest concentration. *C. albican* produced higher DZI (9 mm) than bacterial isolates at the lowest concentration. Also, the Gentamycin

positive control produced incomparably high DZI against the bacterial isolates than the latex of *C. procera.*

Table 2: Antibacterial Activity of *Calotropis procera* Latex on Microbial Isolates

Test Organisms	Zone of Inhibition (mm) for different Concentrations (%(v/v))					
	20	40	60	80	100	GT
Staphylococcus aureus	7.5	10.5	13.5	16.0	19	41
Salmonella typhi	7.0	8.0	9.0	10.0	11.0	40
Escherichia coli	0.0	7.0	8.5	9.5	12.5	41
Candida albican	9.0	10.0	14.0	16.0	18.5	NA

Table 2 shows the average diameter zone of inhibition (DZI) in mm produced by different concentrations of the latex of *C. procera* against *S. aureus, S. typhi, E. coil and C. albican* thus portraying their susceptibility to the latex. GT signifies the Gentamycin antibiotic positive control while NA is 'not applicable' which means no positive control for *C. albican.*

Table 3: Antibacterial Activity of Leaf Extract of *Calotropis procera* on Microbial Isolates

Test Organisms	Zone of Inhibition (mm) for different Concentrations (mg/mL)				
	30	50	70	100	GT
Staphylococcus aureus	00	00	9.0	11.0	42
Salmonella typhi	00	00	7.5	9.0	40
Escherichia coli	10.0	13.5	18.0	20.0	40
Candida albican	12.0	14.0	19.0	21.5	NA

Table 2 shows the average diameter zone of inhibition (DZI) in mm produced by different concentrations of the leaf extract of *C. procera* against *S. aureus, S. typhi, E. coil and C. albican* thus portraying their susceptibility to the latex. GT signifies the Gentamycin antibiotic positive control while NA is 'not applicable' which means no positive control for *C. albican.*

E. coli produced the least susceptibility with DZI of 10 mm by the lowest concentration (30 mg/mL) of the *C. procera* leaf extract while *S. aureus* and *S. typhi* were not susceptible to both 30 and 50 mg/mL of the leaf extract thus resistant. Again, the fungi, *C. albican* was more susceptible than the bacterial isolate at the lowest concentration of 30 mg/mL. Also, the antibiotic used as positive control produced the highest DZI against the three test bacterial isolates.

In this study, MIC and MBC bioassay was carried out using only the latex of *C. procera* as it shows better antibacterial activity against the bacteria test organisms than the leaf extract of *C. procera.* Also, concentrations of 12.5 and 25 % (v/v) inhibited the growth of *S. aureus* and *E. coli* while only the highest concentration of 25 % (v/v) inhibited the growth of *S. typhi* and became the MIC.

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Table 4: Detection of Bacterial Inhibition by *C. procera Latex* on Bacterial Isolates

Bacterial isolates	Concentration (% (v/v))				
	25	12.5	6.3	3.1	1.5
Staphylococcus aureus	-	-*	+	+	+
Salmonella typhi	-*	+	+	+	+
Escherichia coli	-	-*	+	+	+

Table 4 shows the MIC where the negative sign indicates the absence of turbidity in the tube signifying no growth of the test organisms, the positive sign indicates the presence of turbidity in the tube as a result of growth of the test organisms while the negative sign with an asterisk is the MIC which was the lowest concentration that inhibited the growth of the test organisms.

Table 5: Detection of Bactericidal Concentration of *C. procera* Latex against Bacterial Isolates.

Bacterial isolates	Concentration (% (v/v))	
	25	12.5
Staphylococcus aureus	-	+
Salmonella typhi	+	+
Escherichia coli	-	+

Table 5 shows the MBC where the negative signs indicate the absence of colonies or growth of test organisms on the Mueller-Hinton agar plate and designated as the MBC which was the lowest concentration of the *C. procera* latex that kills the test organisms.

Table 5 shows that no bacterial colonies or growth was observed at 25 % (v/v) of *C. procera* latex tested on *S. aureus* and *E. coli*. However, colonies or growth of *S. typhi* were observed for 25 and 12.5 % (v/v) of *C. procera* latex.

Table 6: Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of *C. procera* Latex tested against the Bacterial Isolates.

Bacterial isolates	MIC	MBC
Staphylococcus aureus	12.5	25.0
Salmonella typhi	25.0	-
Escherichia coli	12.5	25.0

Table 6 shows the MIC and MBC of the leaf of *C. procera* latex against *S. aureus, S. typhi* and *E. coli.* Where the hyphen sign (-) implies no MBC detected from the range of concentrations used for the MBC bioassay. MIC and MBC in % (v/v).

MIC means the lowest concentration which inhibits growth (no visual turbidity) of the test organisms (Lajubutu *et al.,* 1995). In this study, 12.5 % (v/v) is the MIC for S. aureus and *E. coli* while 25 % (v/v) became the MIC for *S. typhi* (Table 6). For the MBC, 25 % (v/v) of *C. procera* latex caused bactericidal effects on *S. aureus* and *E. coli*. However, 12.5 and 25 % (v/v) of *C. procera* latex had no bactericidal action on *S. typhi.*

DISCUSSION

In Table 2, the susceptibility of both bacterial and fungal test organisms to various concentrations of *C. procera* latex is evidenced via the production of varied zones of inhibitions, this portrays its antimicrobial activity against the test microorganisms. Furthermore, the zone of inhibition recorded increased as the concentrations of the *C. procera* latex or leaf extract increased and vice versa consequently depicting a concentration-dependent activity (Tables 2 and 3). This phenomenon is reasonably common as also reported in many studies such as Ibrahim *et al.,* (2022) that reported a similar trend of increasing plant extract efficacy with increasing dose or concentration. From Table 3, the leaf extract of *C. procera* also demonstrated some level of antimicrobial activity against the test organisms, especially with *E. coli* which was susceptible to even the lowest concentration (30 mg/mL) of the extract. However, more susceptibility to the latex and the leaf extract of *C. procera* was observed against *C. albican* which is a fungal isolate than its bacterial counterpart as noticed in Tables 2 and 3. Furthermore, the Gentamycin antibiotic used as positive control produces significantly higher antimicrobial activity than both the latex and the leaf extract of *C. procera* (Table 2 and 3). Importantly, the findings of the antimicrobial activity of the latex and leaf extract of *C. procera* in this study corroborate other relevant studies (e.g., Yesmin *et al.,* 2008; Kar *et al.,* 2018; Kareem *et al.,* 2008) that reported the antimicrobial potency of the *C. procera* plants extract. For instance, Salem *et al.,* (2014) reported that both ethanolic and methanolic extracts of the plant extract produced good antimicrobial potency, while the aqueous extract of *C. procera* also had potent antibacterial activity (Panda, 2014).

It should be acknowledged that the phytochemical constituents of the leaf extract and latex of *C. procera* were not investigated in this study. Yet, the antimicrobial activity portrayed by the leaf and latex of *C. procera* against the microbial isolates is majorly attributed to the presence of bioactive phytochemicals, as literature has extensively associated several important phytochemical compounds with *C. procera* plant. The antibacterial actions observed are due to the presence of bioactive secondary metabolites reported as active plant components responsible for activities (Salihu and Garba, 2008). In support of this, Wojdylo *et al.,* (2007) mentioned that bioactive molecules are mostly produced by medicinal plants which reactively inhibit microbial growth thereby rendering protection against infectious disease agents. Phytochemical compounds have enabled various degrees of activity against pathogenic microbes, and are supposed to produce no or lesser side effects as compared with synthetic antimicrobial agents (Konate *et al.,* 2012; Pathania *et al.,* 2020). In specific, tannins possess a mechanism of action which enable them to bind proteins as a result inhibit the cell protein synthesis of microbes (Stern *et al.,* 1996). Also, tannins bind to the cell wall of bacteria thus preventing its growth and the activity of protease, also toxic to filamentous yeast, fungi, and bacteria of ruminal origin (Jones *et al.,* 1994; Oyewole *et al.,* 2004). Chan *et al.,* (2013) and Gupta & Pandey, (2020) expatiate on the role of flavonoids exhibiting inhibitory activity against some resistant microbes via antagonising or reversing their resistance

mechanisms. Furthermore, the direct or indirect destruction of the bacterial cell wall or membrane has been denoted as the common antimicrobial mechanisms of action of flavonoids (Wu *et al.,* 2013; Gupta & Pandey, 2020), and could also inhibit crucial enzymatic pathways of bacteria (Nenaah, 2013). Concerning this study, Nenaah, (2013) isolated four flavonoid compounds (Rutin, (13), kaempferol-3-O-rutinoside (14), isorhamnetin-3-O-rutinoside (15), 5-hydroxy-3,7-dimethoxyflavone-40-O bglucopyranoside (16)) from *C. procera* extract and was tested against fungal isolates and Gram-positive and –negative bacteria) thus reported Rutin (13) as the most effective compound against the bacteria strains with *S. aureus* as one of the most sensitive isolates. These and many more have validated the bioactive phytochemicals as the primary components responsible for the antimicrobial activity of *C. procera.*

Also, proof of the presence of these phytochemicals in the *C. procera* plant and plant extract has been widely reported though not assessed in this study. Mali *et al.,* (2019) reported that *C. procera* contains numerous biologically active chemical compounds or groups namely, saponins cardenolides, tannins, steroids, phenols, glycosides, flavonoids, terpenoids, alkaloids and sugars. Also, the aqueous, methanolic and ethanolic phytochemical screening of *C. procera* root and leaf extract exposed the presence of alkaloids, flavonoids, saponins, tannin and glycosides (Mainasara *et al.,* 2011; Mainasara *et al.,* 2012), however, leaf extract lacks steroids, balsams (Mainasara *et al.,* 2012). To support the antimicrobial activity observed in this study, Kawo *et al.,* (2009) reported the phytochemical result of the ethanolic extract of *C. procera* leaf and latex to contain steroid glycosides, reducing sugar, saponins, tannins, and flavonoids, although alkaloids and resins were absent.

In this study (table 2 and 3), the latex of *C. procera* exhibited better antimicrobial activity against the test organisms than the leaf extract, and this conform with Kareem *et al.,* (2008) which revealed in their study that the latex of *C. procera* demonstrated a stronger inhibitory effect on the test microorganisms than the leaf. Reasonably, this could be attributed to the differences in their bioactive phytochemical components. The variation in the antimicrobial activity observed in this study is in agreement with Duke (1992) and Yusha'u *et al.,* (2008) that reported antibacterial potency or activity may differ according to plant parts (from one part of the plant to another).

As earlier mentioned, the antifungal activity against *C. albican* for both leaf extract and latex of *C. procera* is higher than the antibacterial activity (against bacterial isolates). Kareem *et al.,* (2008) also recorded the best antifungal activity for ethanol extract of *C. procera* latex against *C. albicans* with MIC between 5.0 and 20 mg/mL, though *C. albican* was not inhibited by the aqueous leaf and latex extract of *C. procera.* Again, it must be acknowledged that the MIC of the latex and leaf extract of *C. procera* was not investigated against *C. albican* in this study.

From the MIC result (table 4 and 6), there was variation in the MIC of *C. procera* latex against bacterial isolates, though *S. aureus* and *E. coli* produced the same MIC of 12.5 % (v/v) which suggest that a lower dose of the sap or latex can still inhibit the growth of disease-causing microorganisms thus buttressing on the antibacterial efficacy of the latex. However, *S. typhi* required a higher MIC of *C. procera* latex to inhibit the test organisms. This variation in MIC is expected as Sabo and Knezevic (2019) clarified differences in inhibitory concentrations or MIC could depend on the test microorganism, the plant's antimicrobial properties and the extraction method. This study (table 6) also demonstrated that a

higher concentration of *C. procera* latex is required to cause any bactericidal effects on the bacterial test organisms as MBC was detected at 25 % (v/v) of the latex. Nevertheless, the same concentration of 25 % (v/v) was not bactericidal against the *S. typhi* thus higher dose of the latex could be bactericidal to the organism.

Despite the antimicrobial activity of *C. procera* latex shown in this study, relevant studies (e.g., Lakhtakia *et al.*, 2010; Al-Mezaine *et al*., 2008; Naz *et al*., 2020**;** Mohammad *et al.,* 2021) have elaborated on the toxicity of the plant (especially the latex) specifically the ocular toxicity mainly as a result of the presence of toxic compounds like the toxic cardenolides in the latex and other plant parts (Mohammad *et al.,* 2021).

CONCLUSION

In this study, infectious microbial agents such as *S. aureus, S. typhi, E. coli* and *C. albican* exhibited susceptibility to the varied concentrations of *C. procera* latex and leaf extract though the activity was concentration dependent. The antifungal activity of the latex and leaf extract of *C. procera* was higher than the antibacterial activity as deduced from the DZI produced.

Also, the inhibition of test organisms by the *C. procera* latex at low MIC doses and the bactericidal effect detected at quarter strength of the *C. procera* extract additionally suggests its enormous antimicrobial prospects. These findings buttressed the antimicrobial prowess of *C. procera* latex and leaf extract against the test microorganisms while attributing potency to the surplus bioactive phytochemicals with some reported mechanism of action. By inference, these findings of valuable antimicrobial potency may authenticate its vastly reported age-old traditional medicinal use which is still invoked in today's modern plant medicine. Future studies can elaborately investigate the antifungal ability in terms of the Minimum Fungicidal Concentration (MFC) with diverse fungal isolates and the mechanisms of actions of other phytochemicals.

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