

## Evaluation of phytochemical constituents and *in vitro* antimicrobial activities of leaves extracts of *Calotropis procera* against certain human pathogens

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### Abstract

*Calotropis procera*, a well-known and commonly used plant in Nigeria to treat many infectious diseases, has been documented to possess constituents with proven antimicrobial potentials. The type of solvent used in herbal extraction determines the number of phytoconstituents extracted, therefore it was investigated the effect of some solvents selected based on polarity on the phytoconstituents and antimicrobial efficacy of leaves of *C. procera*. Phytochemical screening of extracts from leaves of *C. procera* obtained from five solvents (acetone, hexane, ethylacetate, mixture “acetone, hexane, ethyl acetate 50/30/20 v/v/v” and sterile distilled water) was carried out. Disc diffusion assay, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimal Fungicidal Concentration (MFC) of the extracts were compared with those of commercial drugs (chloramphenicol, gentamicin and griseofulvin) against *Candida albicans*, *S. aureus* ATCC 25923 and *E. coli* ATCC 25922. Tannins, steroids, alkaloids, saponins, glycosides, terpenoids, proteins, flavonoids, coumarins and anthraquinones were detected in higher numbers in ethyl acetate and sterile distilled water extracts, while the least number of constituents were detected in hexane extracts. A dose- dependent broad spectrum of bacteriostatic/fungistatic and bactericidal activities was produced by the extracts against the tested pathogens. Wider inhibition zone (19.80 mm) was produced at 100 mg/mL concentration by the mixture of solvents and ethylacetate extract, respectively, while the lowest inhibition zone (7.6 mm) was produced by sterile distilled water extract at 25 mg/mL. The present study provided plausible information to corroborate the medicinal value of the investigated plant, as claimed by herbal medicine users.

**Keywords:** antibacterial; antifungal; *C. procera*; phytochemicals; polarities

### Introduction

*C. procera* (Ait) R. Br. Belongs to the Apocynaceae subfamily, formerly in the family Asclepiadaceae (Verma *et al.*, 2010); is a flowering plant of about 2 meters high and 25 cm stem diameter (Little *et al.*, 1974). All parts of the plant secrete white milky sap from cut (Aliyu, 2006). The plant, commonly known as ‘giant-milkweed’, ‘calotrope’, ‘King’s crown’, ‘kapok tree’ is native to tropical Africa, Asia and now introduced to the Southern United States and Brazil (Tennet, 1993; Crothers *et al.*, 1998). It is well-known in Nigeria as

“bomubomu” (Southwestern Nigeria) and “Tumfatiya” (Northern Nigeria, where it is more common) (Chundattu *et al.*, 2012).

Different parts of *C. procera* are used traditionally across many countries (Murti *et al.*, 2010) to treat skin diseases like measles, muscular spasm, stomach ache, peptic ulcer, joint pain and constipation, etc. (Nenaah, 2013). Warmed leaves are used to relieve stomach, back and joint (Amit *et al.*, 2012). Gwari communities of Northern Nigeria also use *C. procera* for topical treatment of ringworms (Kuta, 2008).

Also, antibacterial (Gajare *et al.*, 2012) and antifungal (Kareem *et al.*, 2008; Meena *et al.*, 2010) properties of *C. procera* are well documented. A large number of secondary metabolites have been isolated from this plant (Ashwari, 2009; Morsy *et al.*, 2016). Behl and Luthra (2002) also suggested the presence of active compounds such as alkaloids, steroids in *C. procera* extracts.

Despite the possibility of various solvents to extract numerous constituents from medicinal plants, it is imperative to elucidate the effectiveness of certain solvents based on their polarity in the extraction of constituents from the leaves of *C. procera* and to determine the varying antimicrobial potentials of these solvent extracts on selected pathogenic microorganisms. Therefore, these were the goals of the present investigation.

## Materials and Methods

### *Preparation and extraction of C. procera leaves*

The hereby research was conducted in Al-Hikmah University, Ilorin, Nigeria. Fresh leaves of actively growing *C. procera* plants were hand plucked before sunrise. The samples were identified and authenticated at the Herbarium of the Department of Plant Biology, University of Ilorin, Ilorin and a voucher specimen was deposited (UILH/001/1001). The leaves were thoroughly rinsed under running tap to remove dirt and subsequently rinsed with distilled water to remove all forms of contaminants from the running water. They were finally air-dried under the shade and reduced to a fine powder using an electric blender (Master Chef, Mode MC-BL 1980).

Extraction was made from the powdered leaves using five solvents (acetone, hexane, ethyl acetate, mixture “acetone, hexane, ethyl acetate 50/30/20 v/v/v”) at ratio 1:10 w/v of plant material to solvent (Obeidat *et al.*, 2012). The mixtures were left for 48 hours at room temperature with agitation at regular intervals (Asuzu and Onu, 1994), after which each extract was first filtered through a muslin cloth then the filtrate was further filtered through Whatman No. 1 filter paper (Farnsworth, 1996). The resulting filtrate was subsequently concentrated by evaporation to dryness using a rotary evaporator (Model RE Zhengzhou, Henan). The resulting crude extracts were collected, weighed and packed in sterile- labelled airtight McCartney bottles. They were stored at 4 °C for further use.

### *Preparation of different concentrations*

Concentrations of 100 mg/mL, 50 mg/mL and 25 mg/mL were prepared following the method described by Akujobi *et al.* (2004). A quantity (4 g) of each crude extract was dissolved in 40 mL of 5 % Dimethyl sulfide (5% DMSO mixed with 95% distilled water); this gave the stock solution of 100 mg/ml from which two-fold serial dilutions were made by transferring 5 mL from the stock solution into another tube containing 40 mL of 5% DMSO; this was then mixed thoroughly to obtain a concentration of 50 mg/mL. Another 5 mL was transferred from this tube (containing 50 mg/mL solution) into a third tube containing 40 mL of 5% DMSO and mixed thoroughly to give a concentration of 25 mg/mL. These concentrations were used for further antimicrobial assays.

#### *Testing for sterility of the extracts*

Each crude extract was tested for the absence of contaminants using the method described by Dalitha (2008); 1.0 g of each crude extract was serially diluted into 10 mL Mueller Hinton ((HiMedia) broth generate decreasing concentration up to  $10^{-1}$ , after which the tubes were incubated at 37 °C for 24 hours. Clarity of the broth after 24 hours indicated sterility of the extracts.

#### *Test pathogenic strains*

Three pathogenic strains (*Candida albicans*, *Escherichia coli* ATCC 25920 and *Staphylococcus aureus* ATCC 25923) used in the present study were procured from Microbiology Laboratory of University of Ilorin Teaching Hospital, Ilorin. The bacterial strains were collected on nutrient agar slants, while the fungus was collected on Sabouraud Dextrose Agar slant. The strains were kept in the refrigerator at 4 °C and tested for viability by sub-culturing at 37 °C for 24 hours and 48 h at 37 °C respectively on their respective agar before any susceptibility testing.

#### *Preparation and standardization of inoculum*

Pure isolates of the bacteria and yeast were aseptically transferred to sterile test tubes containing about 6 ml each of Nutrient Broth and Mueller-Hinton Broth (MHB). The approximate cell concentration in the broths was standardized by adjusting their McFarland density using a densitometer to achieve the final concentration of  $1.5 \times 10^8$  cfu/mL CFU/mL of each test pathogen individually. These were incubated overnight at 37 °C and for 48 hours at 30 °C for bacteria and *Candida* respectively (Ochei and Kolhatkar, 2008).

#### *Determination of MIC, MBC and MFC of the crude extracts*

MIC of the crude extracts was determined using the microdilution method of CLSI (2008). Twofold serial dilutions of extracts were prepared directly in a microtiter plate containing Mueller Hinton broth (bacteria) and Sabouraud Dextrose broth (SDA) for (fungus) to obtain various concentrations (100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25 mg/mL). The inoculum (0.1 mL of the 18 hours broth culture) of each of the test organisms that have been adjusted to a turbidity equivalent to 0.5 McFarland standard was introduced to each test tube containing the serially diluted extracts. The positive control, containing the reference drug was used as standard/control. The tubes were covered with cotton wool and incubated for 24 hours at 37 °C. The MIC considered as the tube with the lowest concentration of extract without growth after incubation was taken, and recorded (Moshi *et al.*, 2006).

The MBC and MFC of the solvent extracts against the test pathogens were determined by aseptically sub-culturing with the aid of sterile wire loop from plates of the MIC that showed no growth (i.e. clear tube) of the pathogens on to sterile Muller Hinton agar plates and SDA agar plates for bacteria and yeast respectively. The plates were incubated at 37 °C for 24 hours and 30 °C for 48 hours respectively. The agar plates that showed no microbial growth after 24 hours and 48 hours respectively were taken as the MBC and MFC respectively.

The bactericidal or bacteriostatic effect of each solvent extract against the test pathogen was further determined by using the formula of Valgas *et al.* (2007):

Minimum Bactericidal Concentration / Minimum Inhibitory Concentration

or

Minimum Fungicidal Concentration / Minimum Inhibitory Concentration

If the ratio MBC/MIC = 1 or 2, the effect was considered bactericidal, while if the ratio MBC/MIC = 4 or 16, the effect was considered bacteriostatic (Levinson, 2004; Benjamin *et al.*, 2012).

*Determination of antimicrobial susceptibility of the test pathogens*

Antimicrobial susceptibility test of all the test pathogens was done by Disc diffusion technique as previously described by Moshi *et al.* (2006). The test pathogens were inoculated onto Mueller Hinton and SDA for the disc diffusion assay. The discs (prepared from Whatman No. 1 filter paper, sterilized in an autoclave for 15 min at 15 lbs pressure and allowed to cool) were pre-loaded with an equivalence of 20 µl of the respective concentrations (100 mg/mL, 50 mg/mL and 25 mg/mL) of each solvent extract of leaves of *C. procera*. These were used as the antimicrobial discs and standard commercial discs (Chloramphenicol for Gram-positive bacteria, Gentamicin for Gram-negative bacteria and Griseofulvin for the yeast) were used as positive controls. Negative control discs for organic solvents and distilled water extract were loaded with DMSO and distilled water respectively.

A maximum of three antimicrobial discs of each solvent extract was placed (equal distance from each other) onto plate inoculation with the test pathogen using sterile forceps. Positive and negative control discs were also placed on separate inoculated plates respectively. The treated plates were left on the bench for 1 hour for prior diffusion of the extracts from the discs into the agar medium then incubated for 24 hours at 37 °C for bacteria and for 48 hours at 30 °C for the yeast. The test was carried out for all investigated plant extracts and all test organisms in triplicate. Effectiveness of the solvent extracts was determined by calculating the means and standard deviations ( $\pm$  SD) of the diameters of inhibition zone (mm), measured as clear zones around the discs. The negative control (DMSO and distilled water) revealed no activity.

*The percentage yield of the solvents after extraction*

Extraction yields of 200 g of dry raw powder of leaves of *C. procera* using different solvents were calculated using the formula of Ellof (2004).

Percentage yield (%) = Dry weight of extract  $\times$  100 / Dry weight of plant material

*Phytochemical screening*

The secondary metabolites tested in the five solvent extracts of leaves of *C. procera* were tannins, steroids, alkaloids, saponins, glycosides, terpenoids, proteins, flavonoids, coumarins and anthraquinones. The method described by Harborne (1984) was adopted.

*Statistical analysis*

The *in vitro* experiments were done in triplicates and mean values of each triplicate results were presented.

**Results***The percentage yield of the solvents after extraction*

Extraction yields of 200 g of dry raw powder of leaves of *C. procera* using different solvents are presented in Table 1. The obtained results indicated that the highest yield (14.6%) was obtained from acetone extract, while the least yield (4.6 %) was obtained from distilled water extract.

*Phytochemical compound present in the solvent extracts*

Nine phytoconstituents were detected out of the ten constituents (tannins, steroids, alkaloids, saponins, glycosides, terpenoids, proteins, flavonoids, coumarins and anthraquinones) screened in all the solvent extracts of leaves of *C. procera* (Table 2). The highest number (eight out of the ten screened constituents) of secondary metabolites were detected in ethyl acetate and distilled water extracts, seven constituents were detected in acetone extracts, while the least number (six) of secondary metabolites were detected in hexane extracts.

Steroids, alkaloids and saponins were present in all the solvent extracts tested, while coumarins were absent in all the solvent extracts.

**Table 1.** Percentage yield from different solvent extracts of *C. procera* leaves

Solvent	Yield from dry raw powder (g)	Yield (%)
Acetone	29.2	14.6
Hexane	20.3	10.15
Ethyl acetate	14.8	7.4
Distilled water	12.4	4.6

**Table 2.** Distribution of phytoconstituents from different solvent extracts of *C. procera* leaves

Constituents	Acetone	Hexane	Ethyl acetate	Mixture	Distilled water
Tannins	+	+	+	+	-
Steroids	+	+	+	+	+
Alkaloids	+	+	+	+	+
Saponin	+	+	+	+	+
Glycosides	-	-	-	-	+
Terpenoid	+	+	+	-	+
Protein	+	-	+	-	+
Flavonoid	+	+	+	+	+
Anthraquinones	-	-	+	+	+
Coumarins	-	-	-	-	-

Key: + (Present), - (Absent)

#### *Diameters of inhibition zones of solvent extracts against the pathogens at different concentrations*

The result of the *in vitro* disc diffusion assay showed that all the solvent extracts used in the study exhibited a varying degree of concentration-dependent activities against all tested pathogenic microorganisms (Figure 1, 2 and 3). At 100 mg/mL concentration (Figure 1), the widest inhibition zone (19.80 mm) was produced by the mixture of solvents and ethyl acetate extract, respectively, against *E. coli*, while the lowest inhibition zone (8.12 mm) was produced by distilled water extract against *S. aureus*. Similarly, at 50 mg/mL tested concentrations (Figure 2), the widest inhibition zone (18.00 mm) was produced by the mixture of solvents extract against *E. coli*, while the lowest inhibition zone (8.10 mm) was produced by distilled water extract against *S. aureus*. However, at 25 mg/mL concentration (Figure 3), the widest inhibition zone (18.42 mm) was produced by the mixture of solvents extract against *E. coli*, while the lowest inhibition zone (8.00 mm) was produced by distilled water extract against *S. aureus*.

#### *Diameters of inhibition zones of the controls against the pathogens*

DMSO and distilled water showed no activity against the tested pathogens, while the commercial drugs tested produced activities ranging from 25 mm-30 mm (Figure 4).

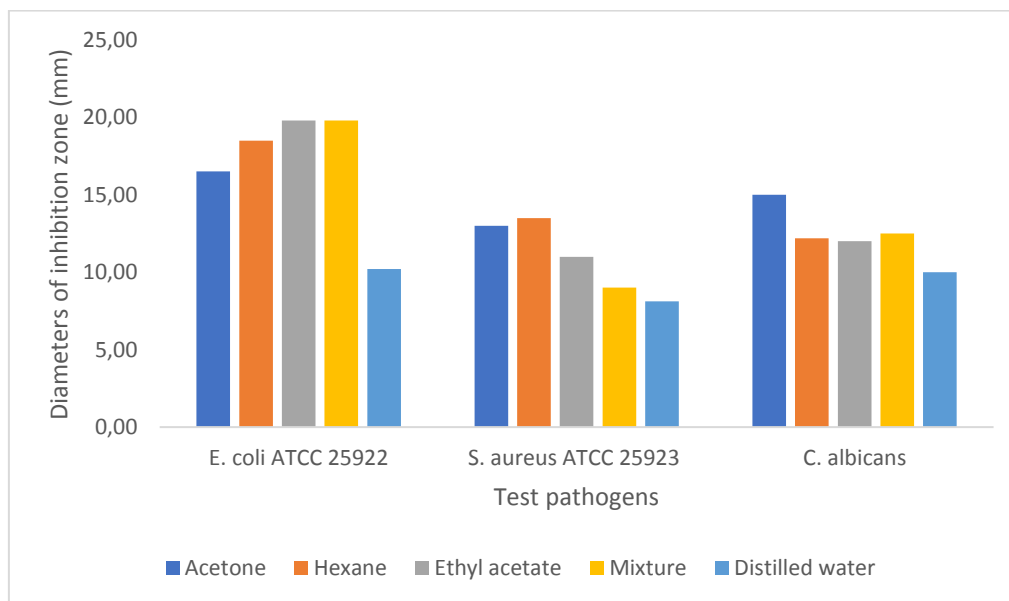
#### *MIC and MBC/MFC values of the solvent extracts against the pathogens at 100 mg/mL*

Varying MIC values were obtained for the solvent extracts against the test pathogens (Table 3). The range of MIC against the pathogens was between 6.25 µg/mL to 100.00 µg/mL. No MIC was obtained by hexane extract on *S. aureus* and none was obtained by distilled water extract against *E. coli*. Generally, the MBC range for all solvent extracts investigated was from 12.50 µg/mL to 100.00 µg/mL.

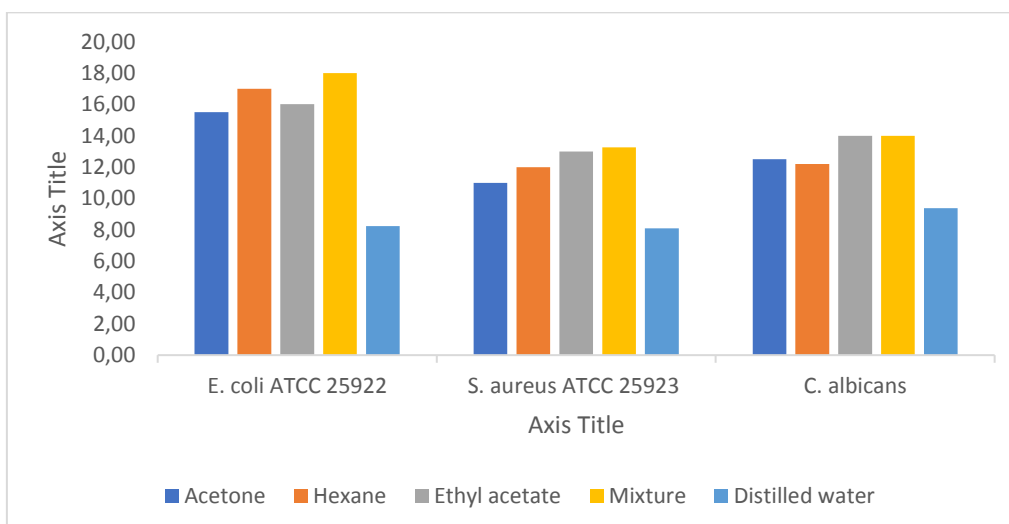
**Table 3.** MIC and MBC/MFC of 100 mg/mL concentration of the solvent extracts of *C. procera* leaves, against specific pathogens

Solvents	MIC ( $\mu\text{g/mL}$ )			MBC/MFC		
	<i>E. coli</i> ATCC 25922	<i>S. aureus</i> ATCC 25923	<i>C. albicans</i>	<i>E. coli</i> ATCC 25922	<i>S. aureus</i> ATCC 25923	<i>C. albicans</i>
Acetone	12.50	50.00	12.50	50.50	100.00	50.00
Hexane	12.50	6.25	12.50	50.00	12.5	25.00
Ethyl acetate	12.50	12.50	25.00	25.50	25.50	50.00
Mixture	6.25	12.50	25.00	12.50	25.00	50.50
Distilled water	-	-	-	-	-	-

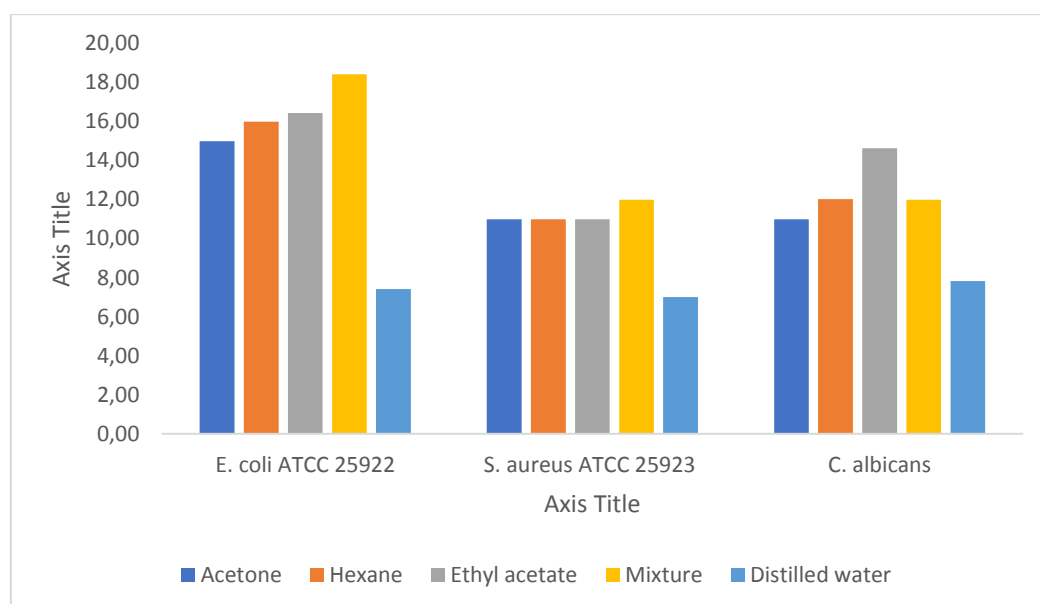
Key: (-) no antimicrobial activity



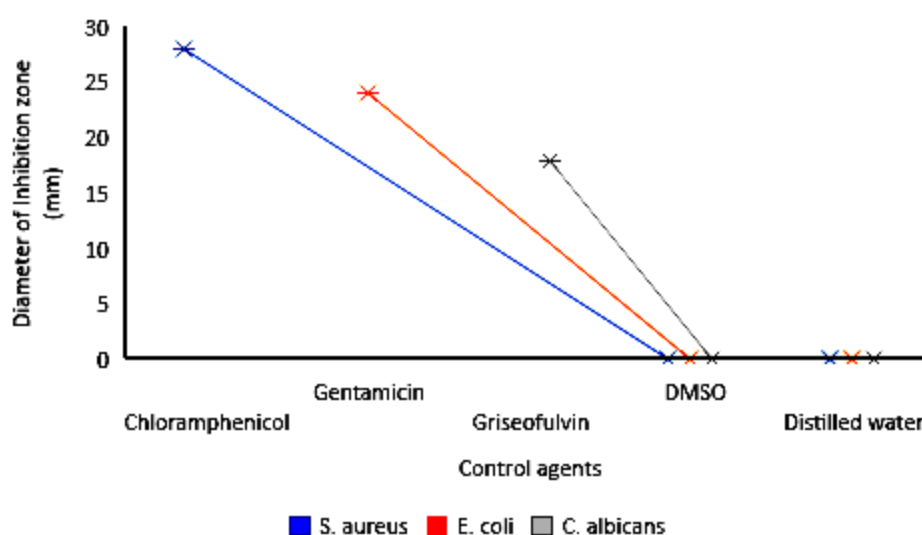
**Figure 1.** Diameters of inhibition zones of *C. procera* extracts at a concentration of 100 mg/mL against the test pathogens



**Figure 2.** Diameters of inhibition zones of *C. procera* extracts at a concentration of 50 mg/mL against the test pathogens



**Figure 3.** Diameters of inhibition zones of *C. provera* extracts at a concentration of 25 mg/mL against the test pathogens



**Figure 4.** Diameters of inhibition zones of control agents against the test pathogens

#### *MIC and MBC/MFC values of the solvent extract against the pathogens at 50 mg/mL*

Table 4 shows the range of MIC at 50 mg/mL against the test pathogens. The value range of MIC against the pathogens was between 6.25 µg/mL to 100.00 µg/mL. Generally, the MBC range for all the solvent extracts was from 12.50 µg/mL to 100.00 µg/mL.

#### *MIC and MBC/MFC values of the solvent extracts against the pathogens at 25 mg/mL*

Table 5 shows the range of MIC at 50 mg/mL concentration against the test pathogens. The MIC values obtained against the test pathogens ranged between 6.25 µg/mL to 100.00 µg/mL. No MIC was obtained for distilled water extract against all the test pathogens. Generally, the MBC range for all the solvent extracts was from 12.50 µg/mL to 100.00 µg/mL.

**Table 4.** MIC and MBC/MFC of 50 mg/mL concentration of the solvent extracts of *C. procera* leaves, against specific pathogens

Solvents	MIC ( $\mu\text{g/mL}$ )			MBC/MFC		
	<i>E. coli</i> ATCC 25922	<i>S. aureus</i> ATCC 25923	<i>C. albicans</i>	<i>E. coli</i> ATCC 25922	<i>S. aureus</i> ATCC 25923	<i>C. albicans</i>
Acetone	12.50	50.00	50.0	50.50	100.00	50.00
Hexane	12.50	100.00	50.00	50.00	100.00	50.00
Ethyl acetate	12.50	25.50	25.00	25.50	25.50	50.00
Mixture	6.25	25.50	25.00	12.50	25.00	50.00
Distilled water	50.00	100.00	100.000	50.00	100.00	100.00

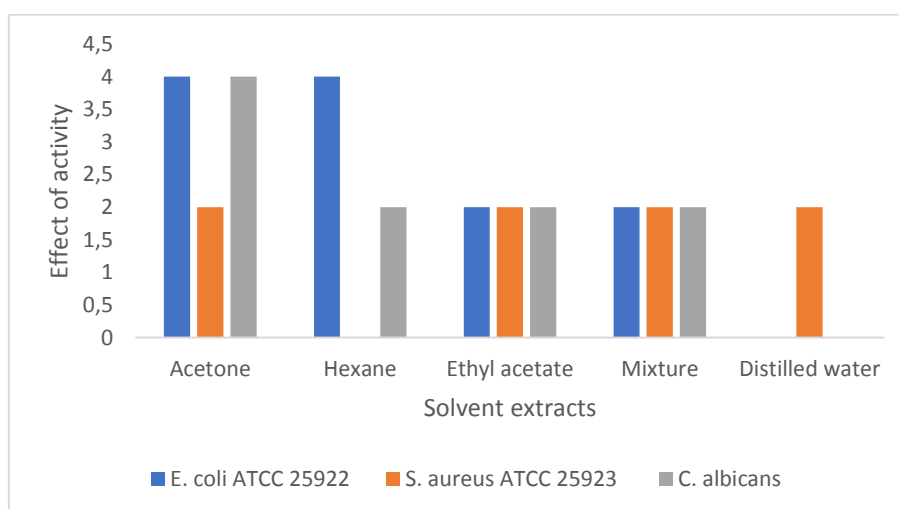
**Table 5.** MIC and MBC/MFC of 25 mg/mL concentration of leaves of *C. procera* leaves, against specific pathogens

Solvents	MIC ( $\mu\text{g/mL}$ )			MBC/MFC		
	<i>E. coli</i> ATCC 25922	<i>S. aureus</i> ATCC 25923	<i>C. albicans</i>	<i>E. coli</i> ATCC 25922	<i>S. aureus</i> ATCC 25923	<i>C. albicans</i>
Acetone	50.00	50.00	100.00	50.00	100.00	100.00
Hexane	25.50	100.00	100.00	50.00	10.0	100.00
Ethyl acetate	12.50	25.00	50.00	25.50	25.00	50.00
Mixture	6.25	25.00	50.00	12.50	25.00	50.00
Distilled water	-	-	-	-	-	-

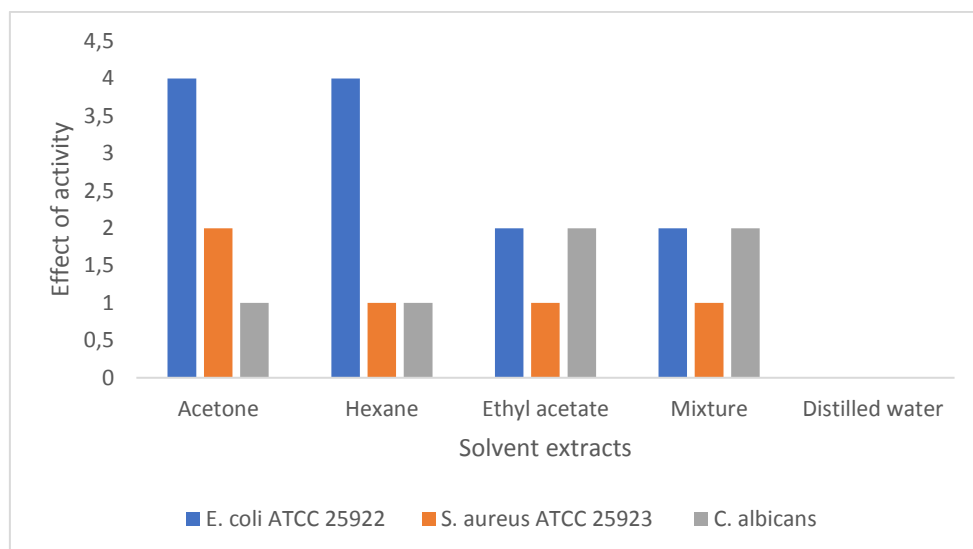
Key: (-) no antimicrobial activity

*Effects of bactericidal/fungicidal and bacteriostatic/fungistatic activities of the solvent extracts*

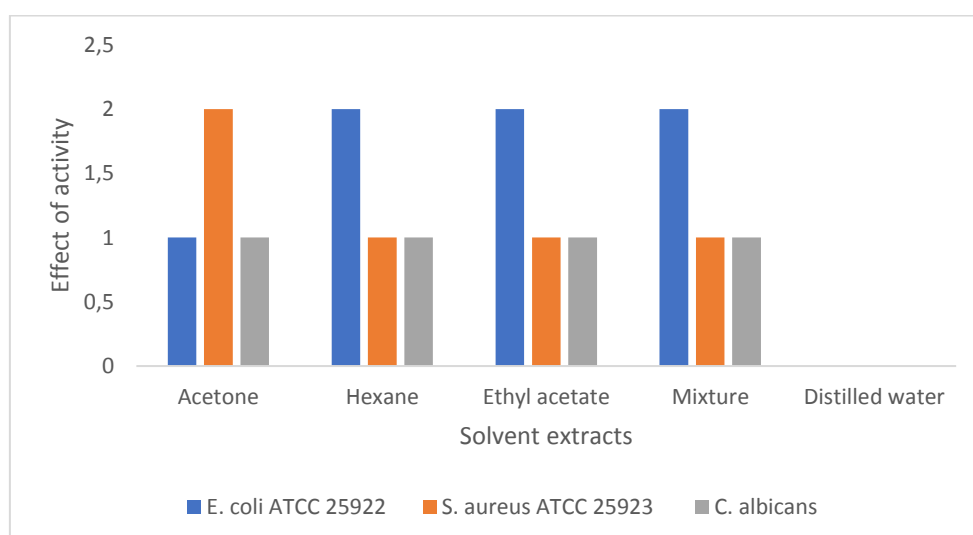
The ratio MBC/MIC (Figure 5, 6 and 7) of the solvent extracts at all the tested concentrations showed a bactericidal/fungicidal effect at 100 mg/mL of acetone (against *E. coli* and *C. albicans*) and hexane (against *E. coli*) and the bacteriostatic and fungistatic effects by other solvent extracts against other tested pathogens (Figure 5). However, bactericidal effect was given by 50 mg/mL of acetone and hexane, respectively, against *E. coli*, while bacteriostatic and fungistatic effect was given by other solvents against other test pathogens (Figure 6). At 25 mg/mL concentration, bacteriostatic and fungistatic effect was given by all the solvent extracts all the test pathogens (Figure 7).

**Figure 5.** The ratio of MBC/ MIC of each solvent extract at 100 mg/mL concentration





**Figure 6.** The ratio of MBC/ MIC of each solvent extract at 50 mg/mL concentration



**Figure 7.** The ratio of MBC/ MIC of each solvent extract at 100 mg/mL concentration

## Discussion

Plants which are regarded as medicinal are known to be rich in a wide variety of secondary metabolites such as tannins, alkaloids, phenolic compounds and flavonoids. The efficacy of these metabolites has been tested in several *in vitro* antimicrobial studies (Djeussi *et al.*, 2013). In the light of the vast traditional use of leaves of *C. procera* and the scientific evidence of the presence of numerous secondary metabolites in the leaves of this plant, the hereby study was aimed at the use of solvents of varying polarities to maximally extract abundant secondary metabolites from the leaves of *C. procera*, and to test for the antimicrobial efficacy of the extracts against pathogenic organisms which are frequently associated with common infectious diseases.

Results of the present study show the presence of several of secondary metabolites including tannins, steroids, alkaloids, saponins, glycosides, terpenoids, proteins, flavonoids and anthraquinones in the solvent extracts of the studied plant. In agreement with the present results is the report of Silva (2010), who stated that *C. procera* is well known for its ability to produce several biologically active compounds. Numerous medicinal

plants have equally been reported to harbor secondary metabolites including tannins, alkaloids, flavonoids and phenolic compounds (Duraipandiyan *et al.*, 2006). The presence of such an impressive number of metabolites as obtained in the current investigation depicts that the leaves of *C. procera* can be used medicinally in the management of ailments caused by pathogenic microorganisms. To buttress this statement is the widely observed and accepted reports that medicinal values of plants is due to the bioactive phytochemicals present in them, that dissolve in different solvent systems (Cowan, 1999).

The presence of tannins only in the alcoholic extracts, but not in the sterile distilled water extracts, may account for the greater antimicrobial efficacy of the alcoholic extracts observed during the *in vitro* assay compared with sterile distilled water extract. A similar observation has been reported by Van Der Watt and Pretorius (2001) on the antibacterial potential of tannins; according to that report, tannins were able to react with proteins to form stable water-soluble compounds, thereby damaging the cell membrane of bacteria, thus lysing them. The presence of alkaloid and flavonoid in all the solvent extracts may also be responsible for their antimicrobial activities. The result is in line with the reports of some authors on the antibacterial activities of alkaloids and flavonoids from *C. procera* (Yesmin *et al.*, 2008).

In general, all the solvent extracts of leaves of *C. procera* showed broad-spectrum antimicrobial activities by producing varying degrees of concentration-dependent inhibition zones against the tested organisms. Various reports have also confirmed that extracts of plants inhibit the growth of various microorganisms at different concentrations (Osadebe and Ukwueze, 2004; Ogueke *et al.*, 2007). Despite the diameters of inhibition zones produced by the solvent extracts (alcoholic and distilled water extracts) against the test pathogens, these amounts were quite inimitable and low, compared to what was produced by the standard drugs of choice against the test pathogens. This may be due partly to the presence of purified compounds in standardized amounts in the standard antibiotics (Gatsing *et al.*, 2010).

Minimum Inhibitory Concentration refers to the lowest concentration of an antimicrobial agent that prevents the growth of a pathogen. High values of MIC are an indication of limited antibacterial efficacy. The present study recorded varying low MIC values among the solvent extracts of *C. procera* against the test pathogens, supporting the antimicrobial activities observed among the solvent extracts against the test pathogens. The results are in line with reports by various researchers that MIC of plants is another important tool in determining the antimicrobial potentials of plants (Gurudeeban *et al.*, 2010; Pavithra *et al.*, 2010).

The acetone and hexane extracts displayed bactericidal activities at 100 mg/mL concentration against Gram-negative organism (*E. coli*), but displayed bacteriostatic/fungistatic against other test organisms. In similarity to the susceptibility of the tested bacteria is the report of Ahmadizadeh *et al.* (2018), which also described the susceptibility of *E. coli* and *S. aureus* to other medicinal plants such as *A. vulgaris*. Of further relevance is the report of Yesmin *et al.* (2008) on the effectiveness of ethanol and water extracts of *C. procera* against both the Gram-positive and Gram-negative bacterial strains (Yesmin *et al.*, 2008). Interestingly too, the report of Farouk *et al.* (2016) stated that the activity of ethyl alcohol extract of leaves of *C. procera* showed activity against both *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 10231 and such data corroborates the observed fungistatic activity of ethyl acetate extract (at 50 mg/mL and 25 mg/mL concentrations) in the present study.

The bactericidal and bacteriostatic/fungistatic activities of the studied solvent extracts may mean that the extracts were able to target the cell wall, disrupt the outer membranes and break the thick peptidoglycan layer for permeability into the bacterial cells, hence, are potent antimicrobial compounds. This is in agreement with individual reports by Cowan (1999) and Hooper (2001), where the mechanisms of actions of antimicrobial agents include targeting of cell wall synthesis, protein synthesis, RNA synthesis, DNA synthesis and intermediary metabolism.

However, the study showed that Gram-negative bacteria (*E. coli*) were more susceptible to the extracts than the Gram-positive bacteria (*S. aureus*). The dissimilarity in the effectiveness of the solvent extracts against Gram-positive and Gram-negative bacteria may be due in part to the difference in morphological composition

of the cells of these organisms, i.e. presence of an outer membrane which covers the cytoplasmic membrane before the peptidoglycan layer, in contrast to the thick peptidoglycan layer on the cell wall of a Gram-positive bacteria (Nikaido, 2003; Wendakoon and Gagnon, 2012).

As to the solvent extracts in general, the most appreciable antimicrobial activity was presented by alcoholic extracts, while distilled water extract exhibited a less interesting activity, with smaller inhibition zone against the test pathogens. However, in contrary to the present finding, other researchers like Yesmin *et al.* (2008) and Mainasara *et al.* (2011) have stated that water extracts of parts of *C. procera* (leaves, flower and fruit) have reported significant activity against both Gram-positive and Gram-negative strains of bacteria. The overall difference in activities of different solvent extracts of *C. procera* used in the study and those previously used by various researchers may be due to the difference in extract compounds. This suggestion was also inferred by Nanasombat (2005).

Variations in the antimicrobial activity of the studied solvents extracts of *C. procera* might be attributed to the high polarity of alcoholic solvents which enhanced their solubility and enabled them to naturally extract higher quantities of metabolites. Among the alcoholic extracts, broader spectrum and highest inhibitory activity were observed with extracts of the mixture of solvents against the test organisms as compared to individual alcoholic solvent. This may be a result of better solubility and improved polarity of the mixed solvents and thus a synergistic extraction of polar and non-polar compounds from the studied plant. Of utmost relevance is the report of Shobowale *et al.* (2015), that noted the solubility and relative polarities of solvents affect the activities of solvent extracts on microbial growth.

## Conclusions

The hereby study provided information on the use of appropriate menstruum in the recovery of abundant secondary metabolites from leaves of *C. procera*. It is also of relevance in the search for alternative chemotherapy, as the study revealed a broad spectrum of action by the extracts obtained from *C. procera* leaves against selected pathogens that are responsible for some infectious diseases, suggesting therefore that the leaves of *C. procera* could be useful solely or as part of preparations of chemotherapeutic agents. However, despite the *in vitro* activity of the solvent extracts tested, such activity may not be translated *in vivo*, hence, the need to further investigate the *in vivo* activities of the solvent extracts of leaves of *C. procera*.

## Acknowledgements

There was no external funding for this research. However, the authors appreciate Mr. Bolu of the Herbarium of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria, for identifying and authenticating the plant.

## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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