Antimicrobial Activity of Extracts and Latex of *Calotropis procera* (Ait.) and Synergistic Effect with Reference Antimicrobials

Gomah E. Nenaah[a *] and Essam M. Ahmed[b]

[a]Department of Zoology, Faculty of Science, Kafrelsheikh University, 33516, Kafr El sheikh, Egypt
* Corresponding author address: Department of Biology, College of Arts and Sciences, Najran University, 1988, Najran, Saudi Arabia. Email: gnenaah2000@yahoo.com
[b]Microbiology Department, Faculty of Medicine, Najran University, Saudi Arabia

Abstract: Aqueous and organic solvent extracts of the leaves, flowers and latex of *Calotropis procera* (Ait.) were tested for their antimicrobial activity using the disc diffusion bioassay. Results revealed a considerable antimicrobial activities of the tested extracts with the extraction solvent was a determinant factor for the extraction of antimicrobial agents. The leaf and latex methanolic extracts showed the strongest activities, where *Escherichia coli, Staphylococcus epidermides,* and *Bacillus spp.* were the most sensitive with inhibition zones reached 23.5 mm and minimal inhibitory concentrations (MIC) between 0.25-1.5 mg/ml. All extracts showed biocidal activities against all of the tested fungal strains with diameters of inhibition zones ranged between 9.0 and 26.5 mm. The latex methanolic was the most effective extract (inhibition zones ranged from 21.0-26.5 mm against *Candida albicans*, *C. tropicalis*, *Penicillium chrysogenum* and *Saccharomyces cerevisiae*). When the latex methanolic extract was added at concentrations equal ½, ¼, ⅛, 1/16 and 1/32 and 0 of the original MIC values, the MIC's of both Ciprofloxacin and Clotrimazole, the two antimicrobial standards, were lowered indicating a synergistic interaction between the botanical and the conventional drugs. Our findings confer the utility of extracts and/or latex of *C. procera* in developing a novel antimicrobial biorationals of plant origin.
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1. Introduction

Despite the fact that new antibiotics are being steadily synthesized through industry, the control of infectious diseases is seriously threatened by the continuous increase in the number of microorganisms that are resistant to the chemical antimicrobial drugs (Cohen 1992; Singer et al. 2003). Such a fact is a cause of great concern, because new multi-resistant bacterial strains are developed, particularly in persons with suppressed immunity. Resistant infections adversely affect mortality, treatment costs, disease spread, and duration of illness (Laxminarayan 2003). Available data also confirmed that resistance has reached unacceptable levels in the pathogens most common in developing countries and that trends show further increases (Okeke et al. 2005). These problems highlights the urgent need for new strategies and new classes of antibiotics (Adcock 2002).

Dependence on plants as a source of medicine is prevalent in developing countries where traditional medicine plays a major role in primary health care. About 80% of individuals from these countries still use plants as remedies from many diseases, using their own personal recipes which have been passed through generations (WHO 2005).

Natural plant products, accordingly provide a continual inspiration of bioactive antimicrobial agents with low toxicity, a broad spectrum and good pharmacokinetics to be clinically used without chemical modification (Silver and Bostain 1990). Therefore, such plants should be investigated to better understand their therapeutic properties, safety and efficiency. Recently there has been a concerted effort to promote the use of botanicals as possible alternatives to treat infectious diseases (Silver and Bostain 1990; Nenaah 2010; Adetutu et al. 2011). These natural products were found to possess promising antimicrobial activities when applied alone or in combination with conventional antimicrobial drugs (Wagner and Ulrich-Merzenich 2009).

Calotropis procera (Ait.) R. Br. (Asclepiadaceae), the so-called "Ushar" is a plant commonly distributed throughout the tropics of Asia, Africa and the Middle East (Singhal and Kumar 2009). The plant is popularly known due to the abundance of latex in its green parts which is easily collected when the plant is wounded. Such a fact reinforces the idea that this milky latex is accumulated as a defense strategy against insects, viruses and fungi (Deepak 1995). Several reports in the literature indicate many therapeutic activities of *C. procera* including analgesic, anti-inflammatory,

cytotoxic, anticancerous and hepatoprotective effects (Dewan et al. 2000; Alencar et al. 2004; Sehgal et al. 2006; Choedon et al. 2006 and Padhy et al. 2007). However little is known about the antimicrobial activities of *C. procera*, except for their activities against a small range of microorganisms (Kareem et al. 2008).

In the present study, we investigate the antibacterial and antifungal activities of different solvent extracts of the leaves, flowers and latex of *C. procera* growing wild in Saudi Arabia when applied alone or in combination with the reference antimicrobial drugs.

2. Material and Methods

2.1. Collection and preparation of the plant sample

The plant *Calotropis procera* was collected from the predesertic region around Najran, KSA during April 2010. A sample of the plant was authenticated by the Botanists of Biology Department, College Arts and Sciences, Najran University, KSA, where a voucher specimen is preserved (voucher no. CpN-01). The leaves and flowers were air-dried for 7 days in the shade at environmental temperature (30-34 ^{o}C day time) and powdered mechanically by using an electric blender (Braun Multiquick Immersion Hand Blender, B White Mixer MR 5550 CA, Germany). Powdered samples were maintained in tightly closed dry bags for subsequent extraction and bioassay.

2.2. Preparation of the test extracts

Five hundred gm of the dry powdered leaves and flowers of *C. procera* were macerated in 5 L capacity glass bottles using distilled water, 80% methanol and diethyl ether (analytical grade, Merck) for 7 days. During this, the samples were periodically shaken for at least 2 h/day using an electric shaker to ensure complete extraction. The extracts were filtered, dried over anhydrous sodium sulphate and reduced under vacuum using a rotary evaporator (Büchi Labortechnike AG, Switzerland) at a temperature not exceeding 65^{o}C. The residues obtained were dried and stored at 4 ^{o}C until bioassayed.

2.3. Collection and preparation of latex extracts

The crude latex was collected from the aerial parts of *C. procera* as described by Singhal and Kumar (2009) with a minor modification. Young leaves near the tip of branches were plucked and the latex that was left to flow was collected in tubes. To prevent natural coagulation, the collected material was gently agitated during collection. It was immediately air dried under shade at ambient temperature with a yield of 20 g per 100ml (20%, DL). To remove the chlorophyll pigments and any rubber materials, the dried latex (DL) was extracted with petroleum ether and filtered. The obtained filtrates were reduced under vacuum and the obtained extracts were, then dried under shade at ambient temperature (32-36 ^{o}C) and collected. Solvent extracts of the dried latex (LD) using distilled water, 80% methanol and diethyl ether (analytical grade, Merck) were prepared as described before and the obtained latex extracts were dried and stored at 4 ^{o}C until bioassayed.

2.4. Test microorganisms

Seven bacterial strains were used in this study. Gram positive bacteria include *Staphylococcus aureus* ATCC 25923, *S. epidermides* ATCC 12228, *Bacillus subtilis* ATCC 6633 and *B. cereus* ATCC 11778. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Streptococcus pneumoniae* ATCC 49619 are the representatives of Gram negative bacteria. In Addition, six different fungal species, *Aspergillus niger, A. flavus, Penicillium chrysogenum, Saccharomyces cerevisiae, Candida albicans* and *C. tropicals* were included.

2.4. Antimicrobial activity bioassay

The antimicrobial activity of the aqueous, methanolic and diethyl ether extracts of the leaves, flowers and latex of *C. procera* against the test microorganisms was determined by using the disc diffusion method (CLSI 2000). All extracts were sterilized through filter sterilization using 0.22 um membrane filter. Sterile filter paper disc (7 mm d) were soaked with the test extract 20 µl and dried at 40 ^{o}C. The prepared nutrient agar plates were seeded with each of the test bacteria (0.10 ml of 10^{7} Cell/ml suspension) and placed on each plate. The test fungi were cultivated on Sabouraud's Dox agar media (5 x 10^{5} CFU/ml) and incubated at 30 ± 2 ^{o}C for 72 h. Ciprofloxacin and Streptomycin discs were used as positive control for bacteria, while Nystatin and Clotrimazole discs were the selected antifungal references. To rule out the activity of the solvent used during the bioassay, solvent-treated discs were prepared and tested as negative control.

2.5. Minimal inhibitory concentrations of the tested extracts

The minimum inhibitory concentrations of the tested botanicals were determined according to a standard procedure (CLSI 2002). Serial dilutions of each of the tested extracts over the range 0.25-6.0 mg/ml were prepared in bacterial broth culture of the tested organisms and incubated at 37 ^{o}C for 24 h for bacteria and in fungi broth media and incubated at 30 ^{o}C for 48 h. The lowest concentration of each extract that inhibits the growth of the tested organism (MIC) was recorded. In addition, the minimal inhibitory concentrations of the antimicrobial standards were determined.

2.6. Evaluation of the synergic interaction between C. procera latex and antibiotics

The Checkerboard agar dilution method was used to evaluate the synergistic effect between *C. procera* latex and the tested antimicrobial standards as reported earlier (Rosato et al. 2007). Eight serial two-fold dilutions of the latex ethanolic extract were prepared as described befor. A series of two-fold serial dilutions of Ciprofloxacin and Clotrimazole, the selected antimicrobial standards, were also prepared. In this way, all antibacterial and antifungal standards dilutions were mixed with the appropriate concentration of the latex thus obtaining a series of combinations of antibiotics and latex. The concentrations prepared corresponded to $^1/_2$, $^1/_4$, $^1/_8$, $^1/_{16}$ and $^1/_{32}$ and 0 of the MIC values. The analysis of the combination of latex/antibiotic combinations was obtained by calculating the FIC index (FICI) as follows: FIC=(MICa of the combination/MICa alone) + (MICb of the combination/MICb alone), where a is either latex extract and b is the standard antibiotic. The FICI was interpreted as follows: (i) a synergistic effect when ≤ 0.5; (ii) an additive or indifferent effect when > 0.5 and <1 and (iii) an antagonistic effect when >1 (Williamson et al. 2001).

2.7. Data analysis

Each experiment of the antimicrobial assessment was set up with six serial dilutions for each compound and then, replicated four times. Results were expressed as means ± S.E. and differences between means were statistically analyzed using an analysis of variance (ANOVA) according to Tukey's HSD test through an SPSS 15.0 software package in Microsoft Widows 7 operating system. Differences are considered significant when $P \leq 0.05$.

3. Results

Results of the present study revealed that *C. procera* extracts showed considerable antibacterial and antifungal activities against the tested microorganisms (Tables 1, 2). In all cases, and regardless of the microorganism tested, the extraction solvent was a determinant factor for the extraction of antimicrobial agents with the methanol was the most effective. In most cases, the latex and leaf methanolic extracts showed the strongest activities. *E. coli* was the most susceptible among the Gram negative bacteria with inhibition zones of 21.5, 18.5 mm with the methanol extracts of latex, and leaves, respectively. Whereas, *P. aeruginosa* and *S. pneumoniae* were more susceptible to the latex methanolic with an inhibition zone of 18.0 mm. In case of Gram positive bacteria, the most potent extract was the latex methanolic with inhibition zones of 23.5, 22.0 and 19.5 mm against *S. epidermides*, *B. subitilis* and *B. cereus*, respectively. However, no antibacterial activity was observed in case of the aqueous extract of leaves and flowers, except for a weak activity against *E. coli* and *S. epidermides*. In this regard, the aqueous extract of the latex showed weak to moderate activities (inhibition zones ranged from 6.5 to 14.0 mm).

All of the test extracts of *C. procera* showed biocidal activities against all of the tested fungal strains. There were significant differences in their activities depending on the microorganism tested and the solvent used with diameters of inhibition zones ranged between 9.0 and 26.5 mm (Table 2). The yeast strains appear to be more susceptible than the mycelial ones with the latex methanolic was the most effective extract (inhibition zones ranged between 21.0 and 26.5 mm). The methanolic extract of the leaves showed considerable activities against all of the tested fungal strains with inhibition zones ranged between 15.0-22.0 mm. Results also revealed that the latex aqueous extract showed promising antifungal activities against *C. albicans* and *P. chrysogenum* with inhibition zones of 20.0 mm.

The MIC values (Tables 3, 4) showed that the lowest values were recorded in case of the leaf and latex methanolic extracts (MIC values of 0.25, 0.50 and 0.75 mg/ml against *E. coli*, *S. epidermidis* and B. *cureus*, respectively for the former and 0.25 and 0.75 mg/ml against *E. coli* and *B. subtilis*, respectively for the later). In case of fungi, the lowest values (0.25-0.750) were recorded in the case of the latex methanolic, where *A. niger*, *C. albicans* and *C. tropicals* were the most sensitive.

The checkerboard micro titer test was employed in our study to explore the possibility of developing more effective combination therapy of *C. procera* latex with the tested antimicrobial standards. In this regard, the MIC values of Ciprofloxacin and Clotrimazole alone were lowered when the latex methanolic extract was added at concentrations equal $^1/_2$, $^1/_4$, $^1/_8$, $^1/_{16}$ and $^1/_{32}$ of the original MIC values (Table 5). The FICI of the latex in combination with Ciprofloxacin against *S. pneumonia*, *S. epidermides*, *E. coli* and *B. cereus* were 0.09 and 0.12, 0.31 and 0.30, respectively. This indicates a synergistic interaction between the botanical and the conventional antibacterial drug at (1/32a+1/16b), (1/16a+1/16b), (1/4a+1/16b) and (1/16a+1/4b) of original concentrations for *S. pneumonia*, *S. epidermides*, *E. coli* and *B. cereus*, respectively. Meanwhile, no synergistic effect was observed in case of *P. aeruginosa* and *S. aureus*. The FICI of Clotrimazole in combination with *C. procera* latex showed a considerable synergism against all of the tested fungi**,** especially in case of *S. cereviciae*, *C. albicans, C. tropicalis* and *P. chrysogenum* at

(1/32a+1/16b), (1/32a+1/8b), (1/32a+1/8b) and (1/16a+1/8b) of original concentrations, respectively.

4. Discussions

According our findings, the aqueous and organic solvent extracts and the latex of *C. procera* showed considerable antibacterial and antifungal activities against the tested microorganisms (Tables 1, 2). In all cases, and regardless of the microorganism tested, the extraction solvent was a determinant factor for the extraction of antimicrobial agents with the latex methanolic extract was the most effective. Among the Gram negative bacteria, *E. coli, P. aeruginosa* and *S. pneumoniae* were the most susceptible strains, while *S. epidermides*, *B. subitilis* and *B. cereus* were the most susceptible among the Gram positive bacterial species (Table 1). Whereas, all the tested extracts, especially the latex methanolic were effective against the test fungal species, especially the yeast ones (Table 2).

In a related study, Kareem *et al.* (2008) concluded that the leaf and latex ethanolic extracts of *C. procera* exhibited moderate antimicrobial effects against *E. coli* (inhibition zone of 14.1 mm). The growth of the tested bacterial isolates were inhibited by the extracts except for *P. aeruginosa* and *S. pyogenes* that were not inhibited by the aqueous extracts. Similarly, the growth of *A. niger, A. flavus, Microsporium boulardii* and *C. albicans* were moderately inhibited by ethanol and chloroform extracts.

Chemically, the latex of *C. procera* is composed of various classes of compounds (Table 6). These were extensively proved in various studies which include proteolytic enzymes, cardenolides, alkaloids, carbohydrates, cardioactive glycoside like calactin, calotropain, proceroside, syriogenine, calotoxin and uscharin, as well as tannins, flavonoids and procerain, a stable cysteine protease (Mossa *et al.,* 1991; Deepak, 1995; Dubey and Jagannadham, 2003). One or more constituents of the latex, separately or in combination, may be responsible for the antimicrobial activity of *C. procera.*

Although reports in the literature indicated several side effects and toxic properties for the latex of *C. procera* such as irritation, inflammation, iridocyclitis and hepatotoxicity (Tomar *et al*, 1970), it was found that oral doses of 0.01 or 0.02 ml/kg body weight of *C. procera* latex were, however, reported non-toxic to sheep and goats (Mahmoud *et al.,* 1979). Likewise, 830 mg/kg body weight oral dose of the dried latex did not produce any toxic effects in mice, where the LD_{50} was found to be 3 g/kg body weight (Dewan *et al.,* 2000). It was found that the dried latex (DL) of *C. procera* did not alter the serum levels of various parameters reflecting liver and kidney functions when orally administered to rats at doses of 10, 100 and 400 mg/kg for a period of 45 days compared to control. Furthermore, no signs of toxicity were observed in the DL treated rats over the study period (Singhal and Kumar, 2009). The author concluded that aqueous suspension of *C. procera* latex does not produce any toxicity and could be safely used for therapeutic purposes at the studied doses. produce any toxicity and could be safely used for therapeutic purposes at the studied doses.

Our study also revealed that, when the latex methanolic extract was added at concentrations equal $^1/_2$, $^1/_4$, $^1/_8$, $^1/_{16}$ and $^1/_{32}$ and 0 of the original MIC values, the MIC's of both Ciprofloxacin and Clotrimazole, the two antimicrobial standards, were lowered indicating a synergistic interaction between the botanical and the conventional drugs. To the best of our knowledge, this is the first report dealing with the interaction between the latex of *C. procera* with the chemical antimicrobial drugs currently in use.

Synergy research in Phytomedicine has established itself as a new key activity in recent years. It is one main aim of this research to find a scientific rational for the therapeutic superiority of herbal drugs derived from traditional medicine as compared with single constituents thereof. Synergy effects of the mixture of bioactive constituents and their byproducts contained in plant extracts are claimed to be responsible for the improved effectiveness of many extracts and conventional antimicrobial drugs (Williamson, 2001; Rosato *et al.,* 2007; Wagner and Ulrich-Merzenich, 2009).

Comparing our results with related studies, Giordani *et al.* (2001) studied the synergistic effect between the latex of *Euphorbia characias* and the antifungal, ketoconazole against *C. albicans*. The authors concluded that the antifungal activity of the chemical drug has been proven to be substantially enhanced at lower concentrations of the latex. The antimicrobial activity of Ciprofloxacin was improved when it was combined to the chloroform leaf extract of *Berberis aetnensis* and tested against *S. aureus* (Musumeci *et al.,* 2003).

Needless to say that, phytochemicals are less potent anti-infectives than conventional antibiotics. Future optimization of these products through structural alteration may allow the development of pharmacologically active agents. It might be possible, for example, to prepare a potent antimicrobial botanical by synthesizing a compound with transformed or substituted ring nucleus. Screening of these analogues might lead to the identification of sufficiently potent biorational antimicrobials. Another approach is the possible application of such biorationals in combined formulations with the conventional antimicrobial drugs.

Based on the findings of the current study, we suggest the combination of latex of *C. procera* and Ciprofloxacin or Clotrimazole for the treatment of bacterial and fungal infections. This may reduce the efficacious doses of these antimicrobials and thus minimize the side-effects of these drugs. The use of therapeutic doses could also be a fix to counter microbial resistance and avoid drug-drug interactions likely to be induced by the administration of currently available antimicrobial drugs. Due to definite allergic reactions that were observed because of the latex of various plant species (Diez-Gomez *et al.,* 1998), further *in vivo* evaluations, including immunocompatibility tests are required before the utilization of the crude latex of *C. procera* in therapeutic applications.

Table (1) Antibacterial activity of *Calotropis procera* extracts against certain pathogenic bacteria using the disc diffusion bioassay

Part used	Extract	% Inhibition zone *(Means ± SE)						
		Gram negative			Gram positive			
		E. coli	*P. aeruginosa*	*S. pneumoniae*	*B. subitilis*	*B. cereus*	*S. aureus*	*S. epidermides*
Leaves	Aqueous	8.0 ± 0.50 ef	na	na	na	na	na	7.5 ± 0.25 f
	Methanol	18.5 ± 0.80 bc	14.0 ± 1.25 c	12.5 ± 0.35 c	16.0 ± 0.55 d	14.5 ± 0.80 d	11.5 ± 0.85 d	13.0 ± 0.40 d
	Diethyl ether	9.0 ± 0.20 ef	7.0 ± 0.45 e	na	6.0 ± 0.50 g	8.0 ± 0.15 f	4.0 ± 0.30 g	5.0 ± 0.10 g
Flowers	Aqueous	6.0 ± 0.10 f	na	na	na	na	na	na
	Methanol	15.5 ± 0.40 c	12.0 ± 0.75 d	10.0 ± 0.85 d	15.0 ± 1.0 de	18.0 ± 0.80 c	10.5 ± 0.75 e	10.0 ± 0.20 e
	Diethyl ether	7.0 ± 0.30 f	7.0 ± 0.40 e	6.0 ± 0.45 e	5.0 ± 0.15 g	6.0 ± 0.20 f	5.0 ± 0.25 g	6.5 ± 035 f
Latex	Aqueous	12.0 ± 0.35 d	6.5 ± 0.40 e	9.5 ± 0.55 d	14.0 ± 0.55 e	10.5 ± 0.35 e	7.0 ± 0.80 f	12.5± 0.70 d
	Methanol	21.5 ± 1.15 b	18.0 ± 0.75 b	11.0 ± 0.45 cd	22.0 ± 1.15 b	19.5± 1.0b c	12.5 ± 0.85 c	23.5 ± 1.25 b
	Diethyl ether	10.5 ± 0.65 de	7.0 ± 0.25 e	7.5 ± 0.30 de	11.0 ± 0.60 f	12.0 ± 0.95 e	9.5 ± 1.0 e	12.5 ± 0.15 d
Streptomycin (200 µg/ml)		19.0 ± 0.25 bc	18.0 ± 0.20 b	18.0 ± 0.40 b	19.5 ± 0.20 c	20.5 ± 0.35 b	14.5 ± 0.45 b	17.0 ± 0.50 c
Ciprofloxacin (60 µg/ml)		28.0 ± 0.20 a	31.0 ± 0.15 a	25.0 ± 0.20 a	31.5 ± 0.10 a	32.0 ± 0.0 a	26.0 ± 0.25 a	30.0 ± 0.35 a
Solvent control		na	na	na	na	na	na	na
**F-values		168	189	169	229	511	618	459

*Values are the mean of four replicates and inhibition zone including the diameter of the bore (7mmd)
In the same column, means followed by the same letters are not significantly different ($P \leq 0.05$); na= not active.
**All F-values are significant at $P \leq 0.001$.

Table (2) Antifungal activity of *Calotropis procera* extracts against certain pathogenic fungi using the disc diffusion bioassay

Part used	Extract	% Inhibition zone *(Means ± SE)					
		Mycelial			Yeast		
		A. niger	*A. flavus*	*P. chrysogenum*	*S. cereviciae*	*C. albicans*	*C. tropicalis*
Leaves	Aqueous	10.0 ± 0.40 e	11.0 ± 1.0 fg	12.5 ± 0.65 ef	12.0 ± 0.45 hi	11.5 ± 0.60 f	14.5 ± 0.80 f
	Methanol	19.5 ± 1.0 b	17.5 ± 0.90 c	18.5 ± 0.55 bc	15.0 ± 0.75 ef	22.0 ± 1.1 c	19.0 ± 0.65 d
	Diethyl ether	12.0 ± 0.35 de	12.5 ± 0.40 ef	15.0 ± 0.55 de	13.0 ± 0.70 gh	12.0 ± 0.75 f	10.5 ± 0.90 g
Flowers	Aqueous	9.0 ± 0.35 e	9.0 ± 0.65 g	10.0 ± 1.1 f	11.5 ± 1.0 hi	10.5 ± 0.95 f	9.5 ± 0.60 g
	Methanol	15.0 ± 1.15 c	15.0 ± 1.0 d	16.0 ± 0.90 cd	16.5 ± 1.25 de	17.5 ± 0.45 d	16.5 ± 0.55 e
	Diethyl ether	12.0 ± 0.85 de	14.0 ± 0.55 de	17.0 ± 0.50 cd	15.0 ± 1.3 ef	10.0 ± 1.0 f	9.5 ± 0.10 g
Latex	Aqueous	11.0 ± 0.65 de	14.5 ± 1.0 de	20.0 ± 1.1 b	14.0 ± 0.85 fg	20.0 ± 2.2 c	16.5 ± 1.25 e
	Methanol	17.5 ± 1.0 b	19.0 ± 1.2 bc	20.5 ± 1.5 d	23.0 ± 1.55 b	26.5 ± 1.35 b	21.0 ± 1.0 b
	Diethyl ether	13.0 ± 1.0 cd	12.0 ± 0.85 fg	17.0 ± 1.0 cd	12.0 ± 0.60 hi	15.0 ± 0.50 e	14.0 ± 0.35 f
Clotrimazole (10 µg/ml)		18.0 ± 0.65 b	21.5 ± 0.45 a	20.0 ± 1.0 b	20.5 ± 0.70 c	25.0 ± 1.0 b	23.5 ± 1.0 b
Nystatin (10 µg/ml)		22.0 ± 0.35 a	22.5 ± 0.50 a	23.5 ± 0.45 a	25.0 ± 0.30 a	35.0 ± 0.25 a	32.0 ± 0.20 a
Solvent control		na	na	na	na	na	na
**F-values		62	88	40	137	314	290

*Values are the mean of four replicates and inhibition zone including the diameter of the bore (7mm).
In the same column, means followed by the same letters are not significantly different ($P \leq 0.05$); na= not active
**All F-values are significant at $P \leq 0.001$.

Table (3): Minimal inhibitory concentrations of *Calotropis procera* extracts against the tested bacterial strains

Part used	Ext	MIC (mg/ml)						
		Ec	*Pa*	*Sp*	*BS*	*Bc*	*Sa*	*Se*
Leaves	Aqueous	5.0 ± 0.15	na	na	na	na	na	6.0 ± 0.25
	Methanol	0.25 ± 0.00	1.5 ± 0.02	2.5 ± 0.08	0.75 ± 0.005	1.5 ± 0.05	2.5 ± 0.05	1.0 ± 0.008
	Diethyl ether	4.0 ± 0.10	4.5 ± 0.15	na	3.5 ± 0.10	5.0 ± 0.15	5.5 ± 0.20	5.0 ± 0.20
Flowers	Aqueous	4.5 ± 0.15	na	na	na	na	na	na
	Methanol	1.5 ± 0.02	2.0 ± 0.05	2.5 ± 0.08	1.5 ± 0.05	2.5 ± 0.05	3.0 ± 0.10	2.0 ± 0.06
	Diethyl ether	3.5 ± 0.10	4.5 ± 0.15	3.5 ± 0.10	4.0 ± 0.15	4.0 ± 0.10	4.5 ± 0.10	3.5 ± 0.10
Latex	Aqueous	4.5 ± 0.15	na	5.5 ± 0.15	5.0 ± 0.15	4.5 ± 0.10	4.0 ± 0.10	4.5 ± 0.15
	Methanol	0.25 ± 0.0	1.5 ± 0.008	1.5 ± 0.05	1.5 ± 0.05	0.75 ± 0.01	3.0 ± 0.10	0.50 ± 0.005
	Diethyl ether	3.0 ± 0.10	3.5 ± 0.15	3.0 ± 0.15	3.5 ± 0.10	3.5 ± 0.10	4.5 ± 0.15	3.5 ± 0.15
Ciprofloxacin (µg/ml)		1.0 ± 0.008	1.5 ± 0.01	2.5 ± 0.05	1.5 ± 0.008	1.5 ± 0.008	2.5 ± 0.10	2.5 ± 0.05
Streptomycin (µg/ml)		10.0 ± 0.10	8.0 ± 0.05	10.0 ± 0.09	10 ± 0.15	10.0 ± 0.10	10.0 ± 0.20	8.0 ± 0.07
Solvent control		na	na	na	na	na	na	na

Ec=Escherichia coli, Pa= Pseudomonas aeruginosa, Sp= Streptococcus pneumoniae,BS= Bacillus subtilis, Bc= Bacillus cereus, Sa= Staphylococcus aureus, Se= Staphylococcus epidermides.

Table (4): Minimal inhibitory concentrations of *Calotropis procera* extracts against the tested fungal strains

Part used	Extract	*MIC (mg/ml)					
		An	*Af*	*Pc*	*Sc*	*Ca*	*Ct*
Leaves	Aqueous	3.0 ± 0.10	3.5 ± 0.10	4.0 ± 0.15	3.0 ± 0.10	2.0 ± 0.08	3.0 ± 0.10
	Methanol	0.75 ± 0.008	1.0 ± 0.005	1.0 ± 0.008	1.5 ± 0.008	0.50 ± 0.002	1.0 ± 0.008
	Diethyl ether	2.0 ± 0.05	2.5 ± 0.05	2.5 ± 0.05	2.5 ± 0.05	3.0 ± 0.10	4.0 ± 0.15
Flowers	Aqueous	3.5 ± 0.10	4.0 ± 0.15	4.0 ± 0.10	3.5 ± 0.10	2.5 ± 0.08	3.0 ± 0.15
	Methanol	1.0 ± 0.05	1.5 ± 0.10	1.0 ± 0.05	1.5 ± 0.10	0.75 ± 0.02	1.5 ± 0.02
	Diethyl ether	3.5 ± 0.10	3.0 ± 0.10	3.5 ± 0.10	3.0 ± 0.15	3.5 ± 0.15	3.0 ± 0.15
Latex	Aqueous	1.0 ± 0.02	1.5 ± 0.05	2.0 ± 0.08	2.0 ± 0.05	0.50 ± 0.008	2.0 ± 0.05
	Methanol	0.25 ± 0.00	1.5 ± 0.02	1.0 ± 0.02	1.5 ± 0.05	0.50 ± 0.005	0.75 ± 0.008
	Diethyl ether	2.5 ± 0.10	2.0 ± 0.02	2.0 ± 0.06	2.5 ± 0.08	2.5 ± 0.05	2.5 ± 0.08
Clotrimazole (µg/ml)		1.5 ± 0.01	2.0 ± 0.05	2.0 ± 0.05	2.0 ± 0.05	1.50 ± 0.02	1.0 ± 0.02
Nystatin (µg/ml)		1.0 ± 0.005	1.0 ± 0.005	1.0 ± 0.005	1.0 ± 0.005	0.50 ± 0.005	0.50 ± 0.005
Solvent control		na	na	na	na	na	na

An= Aspergillus niger, Af =,A. flavus, Pc= Penicillium chrysogenum, Sc= Saccharomyces cerevisiae, Ca= Candida albicans, Ct= C. tropicalis.

Table (5) Fractional inhibitory concentration (FIC) and FIC indices

Microorganism		FIC_a	FIC_b	FICI
Bacteria	*E. coli*	0.25	0.06	0.31
	P. aeruginosa	0.50	0.12	0.60
	S. pneumoniae	0.031	0.062	0.09
	B. subitilis	0.25	0.12	0.40
	B. cereus	0.031	0.25	0.30
	S. aureus	0.25	0.50	0.75
	S. epidermides	0.06	0.06	0.12
Fungi	*A. niger*	0.062	0.25	0.31
	A. flavus	0.12	0.25	0.40
	P. chrysogenum	0.062	0.125	0.19
	S. cereviciae	0.031	0.062	0.09
	C. albicans	0.031	0.12	0.15
	C. tropicalis	0.031	0.12	0.15

FIC_a of latex = MIC of latex alone/MIC of sample in combination.
FIC_b of the standard antimicrobial agents =MIC of standard antimicrobial agents/MIC of the antimicrobial agents in combination.
FIC indices = FIC of Latex + FIC of standard antimicrobial agents.

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Corresponding Author:
Dr. Gomah E. Nenaah
Department of Zoology, Faculty of Science,
Kafrelsheikh University, 33516Kafr el Sheikh, Egypt
E-mail: gnenaah2000@yahoo.com

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