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

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ABSTRACT

The well-documented problems regarding the harmful side effects and the continuous increase in the number of microorganisms that are resistant to the chemical antibiotics highlights the need for new strategies and new classes of antibiotics with low toxicity and high selectivity in their action. In the present study, aqueous and organic solvent extracts of the leaves, flowers and latex of *Calotropis procera* (Ait.) were tested for their antimicrobial activity. For this purpose, the disc diffusion bioassay and the Minimal Inhibitory Concentrations (MICs) of the tested botanicals were adopted. Results revealed considerable antimicrobial activities of the tested extracts. In all cases, 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. In these cases inhibition zones ranged between 11.0 to 23.5 mm and minimal inhibitory concentrations between 0.25-1.5 mg mL^{-1}. 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 to 26.5 mm against *Candida albicans*, *C. tropicalis*, *Penicillium chrysogenum* and *Saccharomyces cerevisiae*). To test any synergistic effect between the latex methanolic and Ciprofloxacin and Clotrimazole, the extract was added to the tested antibiotics at concentrations equal 1/2, 1/4, 1/8 and 1/32 and 0 of the original MIC values. Results revealed that the MIC's of the two antimicrobial standards, were lowered indicating a synergistic interaction between the botanical and the conventional drugs. Present findings confer the utility of extracts and latex of *C. procera* in developing a novel antimicrobial biorationals of plant origin.

Key words: *Calotropis procera*, isolates, antimicrobial biorationals, synergy

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; Jazani *et al*., 2010). 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). Resistance of pathogenic microorganisms to the conventional antibiotics has reached unacceptable levels 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; Rosato *et al.*, 2007). Dependence on plants as a source of medicine is prevalent in developing countries where traditional medicine plays a major role in primary health care (Srivastava *et al.*, 1996). 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 Bostian, 1990). Therefore, such plants should be investigated to better understand their therapeutic properties, safety and efficiency (Eloff, 1998). Recently there has been a concerted effort to promote the use of botanicals as possible alternatives to treat infectious diseases (Cushnie and Lamb, 2005; Mohsenzadeh, 2007; Jazani *et al.*, 2009; Vaghasiya and Chanda, 2010; Nenaah, 2010; Teng *et al.*, 2010; Chanda *et al.*, 2011; Adetutu *et al.*, 2011). These natural products were found to possess promising antimicrobial activities when applied alone or in combination with conventional antimicrobial drugs (Williamson, 2001; Jazani *et al.*, 2007; Rosato *et al.*, 2007; Wagner and Ulrich-Merzenich, 2009). There are a number of herbs for which the medicinal value is still to be investigated so that they can replace and used as an alternate of synthetic drugs (Karim *et al.*, 2011).

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, antidiabetic, cytotoxic, anticancerous and hepatoprotective effects (Dewan *et al.*, 2000; Alencar *et al.*, 2004; Sehgal *et al.*, 2006; Choedon *et al.*, 2006; 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 (Jain *et al.*, 1996; 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.

MATERIALS AND METHODS

Collection and preparation of the plant sample: The plant *Calotropis procera* was collected from the predesertic region around Najran city, 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 had been preserved (voucher No. CpN-01). The leaves and flowers were air-dried for 7 days in the shade at environmental temperature (30-34°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.

Preparation of the test extracts: Five hundred gram 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^{-1} 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 (Bβchi Labortechnike AG, Switzerland) at a temperature not exceeding 65°C. The residues obtained were dried and stored at 4°C until bioassayed.

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 100 mL (20%, Dried Latex, 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°C) and collected. Solvent extracts of the Dried Latex (DL) 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°C until bioassayed .

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.

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°C. The prepared nutrient agar plates were seeded with each of the test bacteria (0.10 mL of 10^7 Cell mL^{-1} suspension) and placed on each plate. The test fungi were cultivated on Sabouraud's Dox agar media (5×10^5 cfu mL^{-1}) and incubated at 30±2°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.

Minimal inhibitory concentrations of the tested extracts: The minimum inhibitory concentrations of the tested botanicals were determined according to a standard procedure (CLSI, 2002; Eloff, 2004). Serial dilutions of each of the tested extracts over the range 0.25-6.0 mg mL^{-1} were prepared in bacterial broth culture of the tested organisms and incubated at 37°C for 24 h for bacteria and in fungi broth media and incubated at 30°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.

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 (White *et al.*, 1996; Rosato *et al.*, 2007). Eight serial two-fold dilutions of the latex ethanolic extract were prepared as described before. 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 and1/32 and 0 of the MIC values. The analysis of the combination of latex/antibiotic combinations was obtained by calculating the Fractional Inhibitory Concentration Index (FICI) as follows: FICI = (MICa of the combination/MICa alone)+(MICb of the combination/MICb alone), where MICa and MICb are the minimal inhibitory concentrations of the latex (a) and the test antibiotic (b), respectively. The FICI was interpreted as follows: (1) a synergistic effect when = 0.5; (2) an additive or indifferent effect when>0.5 and<1 and (3) an antagonistic effect when>1 (Williamson, 2001).

Data analysis: Each experiment 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 One way analysis of variance according to Tukey's HSD test through an SPSS 15.0 software package in Microsoft Widows 7 operating system. Differences are considered significant when $p \leq 0.05$.

RESULTS

Results of the present study revealed that *C. procera* extracts showed considerable antibacterial and antifungal activities against the tested microorganisms (Table 1, 2). The extraction solvent was a determinant factor for the extraction of antimicrobial agents, regardless of the microorganism tested. In this regard, 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 inhibition zones of 18.0 and 11 mm, respectively. 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.

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

		% Inhibition zone *(Mean±SE)						
		Gram negative				Gram positive		
Part used	Extract	*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±0.30g	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^{-1})		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^{-1})		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 (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 2: Antifungal activity of *Calotropis procera* extracts against certain pathogenic fungi using the disc diffusion bioassay

		% Inhibition zone *(Means±SE)					
		Mycelial			Yeast		
Part used	Extract	*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}	15.00.50^{e}	14.0±0.35^{f}
Clotrimazole (10 µg mL^{-1})		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^{-1})		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 $p \leq 0.001$

The MIC values (Table 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^{-1} against *E. coli*, *S. epidermidis* and *B. cureus*, respectively for the former and 0.25 and 0.75 mg mL^{-1} against *E. coli* and *B. subtilis*, respectively for the later). In case of fungi, the lowest values (0.25-0.750) were recorded with the latex methanolic, where *A. niger*, *C. albicans* and *C. tropicals* were the most sensitive.

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

		MIC (mg mL^{-1})						
Part used	Extract	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±0.008	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})		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^{-1})		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*, Na: Not active

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

		*MIC (mg mL^{-1})					
Part used	Extract	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})		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})		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*, Na: Not active

The checkerboard micro titer test was employed in present 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 and1/32 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

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

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.

DISCUSSION

According the findings of the present study, the aqueous and organic solvent extracts and the latex of *C. procera* showed considerable antibacterial and antifungal activities against the tested microorganisms (Table 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).

Our results are in accordance with those of Yesmin *et al.* (2008) who concluded that crude methanol extract of *C. procera* at a concentration of 500 μg mL^{-1} showed moderate antibacterial activities using the agar well diffusion bioassay *S. aureus, S. epidermidis, Plesiomonas shigelloides, Shigella dysenteriae* and *Vibrio cholera*. On the other hand, aqueous extract at the same concentration was effective against *Staphylococcus aureus, Staphylococcus, epidermidis, Staphylococcus saprophyticus, Streptococcus pyogenes Plesiomonas shigelloides, Shigella dysenteriae, Vibrio cholerae, Shigella Flexner, Shigella sonnei* and *Pseudomonas aeruginosa*. In that study, diameter of inhibition zones were ranged between 6 and 22 μg $well^{-1}$. In a study conducted by Kareem *et al.* (2008), the leaf and latex ethanolic extracts of *C. procera* exhibited moderate antimicrobial effects against *E. coli* (inhibition zone of 14.1 mm). The growths of the tested bacterial isolates were inhibited by the extracts except for *P. aeruginosa* and

S. pyogenes. Similarly, the growth of *A. niger, A. flavus, Microsporium boulardii* and *C. albicans* were moderately inhibited by ethanol and chloroform extracts.

In a study conducted by Kawo *et al.* (2009), a weak antibacterial properties of the ethanolic extracts of the leaves and latex of *C. procera* against *E. coli, S. aureus, Salmonella species* and *Pseudomonas species* was recorded by using paper-disc diffusion and broth dilution techniques. The results obtained revealed that ethanol was the best extractive solvent for a fraction with antibacterial activity. Generally, the aqueous extracts showed no activity on the isolates. The Minimum Inhibitory Concentration (MIC) for the leaf ethanolic extract was 1000-2000 μg mL^{-1}, while the Minimum Bactericidal Concentration (MBC) of the latex ethanolic was 2000 μg mL^{-1}.

Chemically, the latex of *C. procera* is composed of various classes of phytochemical compounds. These were extensively proved in various studies which include proteolytic enzymes, cardenolides, alkaloids, 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* (Deepak, 1995; Dubey and Jagannadham, 2003).

Although reports in the literature indicated several side effects and toxic properties for *C. procera* latex like. These include irritation, inflammation and hepatotoxicity (Tomar *et al.*, 1970). It was found that oral doses of 0.01 or 0.02 ml kg^{-1} body weight of *C. procera* latex were, however, reported non-toxic to sheep and goats (Mahmoud *et al.*, 1979). A dose of 830 mg kg^{-1} 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^{-1} body weight (Dewan *et al.*, 2000). The Dried Latex (DL) of *C. procera* did not alter the liver and kidney functions when orally administered to rats at doses of 10, 100 and 400 mg kg^{-1} for a period of 45 days compared to control (Singhal and Kumar, 2009). The author stated that aqueous suspension of *C. procera* latex does not 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 and1/32 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).

CONCLUSION

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 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. It was reported that definite allergic reactions were observed because of the latex of various plant species including *C. procera* (Diez-Gomez *et al*., 1998). Further *in vivo* evaluations, including immunocompatibility tests are, however, required before the utilization of crude latex of *C. procera* in therapeutic applications.

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