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Identification of the Bioactive Constituents and the Antibacterial, Antifungal and Cytotoxic Activities of Different Fractions from *Cestrum nocturnum* L.

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Abstract

This study was carried out to evaluate the antibacterial, antifungal and cytotoxic effects of chloroform, ethyl acetate and aqueous fractions obtained from a methanol 80 % extract of *Cestrum nocturnum* L. (family Solanaceae) aerial parts, and to investigate the bioactive constituents present in these fractions. The results showed that the chloroform fraction had the highest antibacterial effect and cytotoxicity against lung, colon and hepatocellular carcinoma cell lines, while the ethyl acetate fraction displayed the highest antifungal activity and cytotoxicity against cervical carcinoma cell lines. Phytochemical analysis showed the presence of triterpenes, coumarins and flavonoids in the chloroform fraction. Flavonoids, triterpenes and carbohydrates in the ethyl acetate fraction while tannins, flavonoids, saponins, carbohydrates and triterpenes were detected in the aqueous fraction. *β*-sitosterol, stigmasterol, protocatechuic acid and apigenin were isolated from the chloroform fraction; Kaempferol 8-*O*-methyl ether, kaempferol, kaempferol 3-*O*-α-rhamnoside and luteolin 7-*O*-*β*-glucoside were isolated from the ethyl acetate fraction, while kaempferol di-sugars: kaempferol 3-*O*-*β*-glucoside-7-*O*-α-rhamnoside and kaempferol 3,7-di-*O*-α-rhamnoside, were isolated from the aqueous fraction. This study proves the bioactivities of *C. nocturnum* aerial parts that can be explored as a source of medicinal compounds.

Keywords: *Cestrum nocturnum* L.; Aerial parts; Antimicrobial activity; Cytotoxicity; Phytochemical constituents.

1. Introduction

There is an urgent need to discover new antimicrobial and antitumor agents following the discovery of many infectious diseases (Hawkey, 2008; Jemal *et al*., 2011). Phytochemical screening of various plants revealed that secondary metabolites might be bioactive compounds with different properties such as antibacterial, antifungal, antioxidant, antidiabetic and anticancer (Gurib-Fakim, 2006; Phillipson, 2007; Alonso-Castro *et al.*, 2011). Therefore, compounds or extracts from the plant origin should be explored for the development of new formulations against different diseases.

Cestrum nocturnum L. is an evergreen shrub from the family Solanaceae that grows in tropical and sub-tropical regions throughout the world (Roig, 1988). Leaves of *C. nocturnum* were used to treat burns and swellings (Perez-Saad and Buznego, 2008). The volatile oil was used to prevent malaria (Mimaki *et al*., 2006). Leaves had significant analgesic and bactericidal effects (Huang *et al*., 2006). The inhibitory effects on the central nerve system and cardiac arrhythmic effect of this plant are also documented (Zeng *et al*., 2002). Polysaccharides extracts of *C. nocturnum* had antitumor effects (Zhong *et al*., 2008). Also the whole plant showed an antimicrobial activity against pathogenic microorganisms (Khan *et al.*, 2011). The essential oil from the plant showed an inhibition of some plant pathogens (Al-Reza *et al*., 2009a), and inhibitory effects on food-borne pathogens (Al-Reza *et al.*, 2009b). Its chloroform and toluene extracts gave behavior and analgesic effects (Rodriguez *et al*., 2005). Moreover, *C. nocturnum* leaves had calcinogenic glycoside (Mello, 2003), nocturnoside A and nocturnoside B (Ahmad *et al*., 1995), phenol glucosides (Sahai *et al*., 1994), flavonol glycosides and steroidal saponins (Mimaki *et al*., 2001; Mimaki *et al.*, 2002) and also some recent studies have been done on the plant (Doshi and Mukadam 2016, Chaskar *et al*. 2017). In the present study, the antibacterial, antifungal and cytotoxic activities of different fractions obtained from *C. nocturnum* aerial parts were evaluated. Furthermore, a phytochemical characterization of each fraction was carried out in order to

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understand the compounds involved in each individual fraction bioactivity.

2. Material and Methods

2.1. Equipments, Materials and Chemicals

UV/VIS: Shimadzu UV-visible recording spectrophotometer model-UV 240 (NRC, Egypt). Spectroscopic data (^{1}H-NMR, ^{13}C-NMR): Varian Unity Inova. MS (Finnigan MAT SSQ 7000, 70 ev). Silica gel (60-200 mesh, Merck) for column chromatography, Thin Layer Chromatography (TLC): pre-coated sheets of silica gel 60 F_{254} (Merck). Polyamide powder of caprolactam type (MN-polyamide SC6, Macherey Nagel, Pharmacia Fine Chemicals) for column chromatography, Paper Chromatography (PC), Whatman No.1 (Whatman Led. Maid Stone, Kent, England) sheets. Sephadex LH-20 (Sigma). Solvent mixtures, BAW (n-butanol: acetic acid: water 4:1:5 upper phase, 15% acetic acid: water: glacial acetic acid: 85:15).

2.2. Plant Material

Cestrum nocturnum aerial parts were collected from the Agricultural Research Centre, Giza, Egypt in May 2011 during flowering and identified by Dr. Mohammed El-Gebaly, Department of Botany, National Research Centre (NRC) and by Mrs. Tereez Labib, Consultant of Plant Taxonomy at the Ministry of Agriculture and director of Orman botanical garden, Giza, Egypt. A voucher specimen is deposited in the herbarium of Agricultural Research Centre, Giza, Egypt.

2.3. Preparation of the Plant Extracts

Air-dried powder from the aerial parts of *C. nocturnum* (680 g) was extracted with methanol 80 % several times at room temperature. The extract was concentrated to dryness by rotary evaporator giving 35 g of the crude extract. The extract was fractionated with chloroform, ethyl acetate solvents. The solvent of each partition was evaporated to dryness using rotary evaporator resulting in products weighing 9.5 g, 7 g and 16.5 g. of chloroform, ethyl acetate and water extraction, respectively. Each fraction was tested for the presence of bioactive compounds by using the following standard tests: Molisch test for carbohydrates, Shinoda test for flavonoids, Forth test for saponins, Salkowski for terpenes and sterols, $FeCl_3$ and Mayer's reagents for the detection of tannins and alkaloids, respectively (Harborne, 1973; Trease and Evans, 1989; Sofowra, 1993).

2.4. Antibacterial Activity

The following Gram (-) (*Enterobacter cloacae* human isolate, *Escherichia coli* ATCC 35210, *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella typhimurium* ATCC 13311) as well as Gram (+) bacteria (*Bacillus cereus* clinical isolate, *Listeria monocytogenes* NCTC 7973, *Micrococcus flavus* ATCC 10240 and *Staphylococcus aureus* ATCC 6538) were used. These bacteria were obtained from Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", University of Belgrade, Serbia. The antibacterial assay was carried out by microdilution method (Hanel and Raether, 1988; Espinel-Ingroff, 2001). The bacterial suspensions were adjusted with sterile saline to a concentration of 1.0×10^5 CFU/mL. The inocula were prepared daily and stored at 4°C until use. Dilutions of the inocula were cultured on solid medium to verify the absence of contamination and to check the validity of the inoculum. The minimum inhibitory and bactericidal concentrations (MICs and MBCs) were determined using 96-well microtiter plates. The bacterial suspension was adjusted with sterile saline to a concentration of 1.0×10^5 CFU/mL. The fractions of the same samples (three samples repeated three times) and three extract samples were collected for testing (1 and 10 mg/mL) in broth.

Tryptic Soy Broth (TSB) medium (100 µL) with bacterial inoculum (1.0×10^4 CFU per well) to achieve the wanted concentrations. The microplates were incubated on rotary shaker (160 rpm) for twenty-four hours at 37°C. The following day, 30 µL of 0.2 mg/mL solution of INT (*p*-iodonitrotetrazolium violet) were added, and the plates were returned to the incubator for at least one-half hour to ensure an adequate color reaction. Inhibition of growth was indicated by a clear solution or a definite decrease in color reaction. The lowest concentrations without visible growth (under the binocular microscope) were defined as concentrations that completely inhibited bacterial growth (MICs). The MBCs were determined by serial sub-cultivation of 2 µL into microtiter plates containing 100 µL of broth per well and further incubation for twenty-four hours. The lowest concentration with no visible growth was defined as the MBC, indicating 99.5 % killing of the original inoculum. The optical density of each well was measured at a wavelength of 655 nm by microplate manager 4.0 (Bio-Rad Laboratories), and was compared with a blank and the positive control. The antibiotics streptomycin and ampicillin were used as positive controls (1 mg/mL in sterile physiological saline). Three independent experiments were performed in triplicate.

2.5. Antifungal Activity

The used fungi; *Aspergillus fumigatus* (ATCC 1022), *Aspergillus versicolor* (ATCC 11730), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus niger* (ATCC 6275), *Trichoderma viride* (IAM 5061), *Penicillium funiculosum* (ATCC 36839), *Penicillium ochrochloron* (ATCC 9112) and *Penicillium verrucosum* var. *cyclopium* were obtained from Mycology Laboratory, Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", University of Belgrade, Serbia. The Micromycetes were maintained on malt agar and the cultures were stored at 4° C and sub-cultured once a month. The antifungal assay was carried out by a modified microdilution technique (Hanel and Raether, 1988; Espinel-Ingroff, 2001). The fungal spores were washed from the surface of agar plates with sterile 0.85 % saline containing 0.1 % Tween 80 (v/v). The spore suspension was adjusted with sterile saline to a concentration of approximately 1.0×10^5 in a final volume of 100 µL per well. The inocula were stored at 4° C for further use. Dilutions of the inoculum were cultured on solid malt agar to verify the absence of contamination and to check the validity of the inoculum. MIC determinations were performed by a serial dilution technique using 96-well microtiter plates. The examined fractions of the same sample (repeated three times) were diluted in 5 % of

DMSO (1 mg/mL and 10 mg/mL) and added in broth malt medium (MA) with inoculum. The microplates were incubated in a rotary shaker (160 rpm) for seventy-two hours at 28° C. The lowest concentrations without visible growth (at the binocular microscope) were defined as MICs. The fungicidal concentrations (MFCs) were determined by serial subcultivation of 2 µL of tested fractions dissolved in medium and inoculated for seventy-two hours, into microtiter plates containing 100 µL of broth per well and further incubation for seventy-two hours at 28° C. The lowest concentration with no visible growth was defined as MFC indicating 99.5 % killing of the original inoculum. The fungicides bifonazole and ketoconazole were used as positive controls (1-3500 µg/mL). Three independent experiments were performed in duplicate.

2.6. Cytotoxicity in Human Tumor Cell Lines and in Liver Primary Cell Culture

Five human tumor cell lines were used: MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), HCT-15 (colon carcinoma), HeLa (cervical carcinoma) and HepG2 (hepatocellular carcinoma). Cells were routinely maintained as adherent cell cultures in RPMI-1640 medium containing 10 % heat-inactivated fetal bovine serum (FBS) and 2 mM glutamine (MCF-7, NCI-H460 and HCT-15) or in DMEM supplemented with 10 % FBS, 2 mM glutamine, 100 U/mL penicillin and 100 mg/mL streptomycin (HeLa and HepG2 cells), at 37 ºC, in a humidified air incubator containing 5 % CO_2. Each cell line was plated at an appropriate density (7.5×10^3 cells/well for MCF-7, NCI-H460 and HCT-15 or 1.0×10^4 cells/well for HeLa and HepG2) in 96-well plates. Sulforhodamine B assay was performed according to a procedure previously described by Guimarães *et al*., (2013).

For the hepatotoxicity evaluation, a cell culture was prepared from a freshly-harvested porcine liver obtained from a local slaughter house, according to a procedure established by Guimarães *et al*. (2013); it was designed as PLP2. Cultivation of the cells was continued with direct monitoring every two to three days using a phase contrast microscope. Before confluence was reached, cells were subcultured and plated in 96-well plates at a density of 1.0×10^4 cells/well, and cultivated in DMEM medium with 10 % FBS, 100 U/mL penicillin and 100 µg/mL streptomycin. Three independent experiments were performed in triplicate, and the results were expressed as mean values ± standard deviation (SD).

2.7. Phytochemical Characterization of C. nocturnum Fractions

2.7.1. Phytochemical Analysis of Chloroform Fraction

It was subjected to silica gel column chromatography using chloroform ($CHCl_3$) as eluent and a gradually increasing amount of methanol (MeOH). Four compounds were isolated. Compound one (see figure 3) isolated from $CHCl_3$:MeOH (98:2) elution, and compound two was isolated through elution with $CHCl_3$:MeOH (96:4). Compound three was isolated through elution with $CHCl_3$: MeOH (90:10), and then compound four was isolated through elution with $CHCl_3$: MeOH (85:15).

2.7.2. Phytochemical Analysis of Ethyl Acetate Fraction

It was subjected to silica gel column chromatography using $CHCl_3$ as eluent and a gradually increasing amount of ethyl acetate (EtOAc) and MeOH. Compound five was isolated from $CHCl_3$: EtOAc (50:50) elution, compound six was isolated from EtOAc elution and compounds seven and eight were isolated through further elution with EtOAc and MeOH, gradually.

2.7.3. Phytochemical Analysis of Aqueous Fraction

It was subjected to polyamide column chromatography using distilled water and gradually increasing amounts of MeOH. Compounds nine and ten were isolated through this elution. All the compounds were purified on sephadex LH–20 column which was eluted with mixtures of methanol with distilled water.

2.7.4. Acid Hydrolysis of Flavonoid Glycosides

Solutions of 5 mg of compounds 7, 8, 9 and 10 in 5 ml 10% HCl were heated for five hours. The aglycones were extracted with EtOAc and identified by co-TLC with authentic standards. The sugars in the aqueous layer was identified by co-paper chromatography (co-PC) with authentic markers on Whatman No. 1 sheets in solvent system (*n*-BuOH-AcOH-H_2O 4:1:5 upper layer).

3. Results and Discussion

The obtained results for the antibacterial and antifungal activity are presented in Tables 1 and 2, Figures 1 and 2, respectively. The chloroform fraction showed activity against all the tested bacteria (MIC 0.6-2.5 mg/mL and MBC 1.25-5.0 mg/mL); ethyl acetate fraction exhibited MICs of 1.25-3.75 mg/mL and MBCs of 2.5-7.5 mg/mL, and the aqueous fraction revealed MICs of 0.6-1.25 mg/mL and MBCs of 1.25-5.0 mg/mL. It can be observed that the chloroform fraction gave the highest antibacterial activity, except for *L. monocytogenes* and *E. cloacae*, where the aqueous fraction showed a higher effect. The least effective was the ethyl acetate fraction. The most sensitive bacteria were *E. cloacae*, while the most resistant one was *P. aeruginosa*, followed by *L. monocytogenes*.

All the fractions were more active against microfungi (Table 2 and Figure 2). The chloroform fraction inhibited all microfungi at 0.15-0.6 mg/mL and completely stopped the growth at 0.3-1.25 mg/mL. The ethyl acetate fraction showed inhibitory concentrations at 0.075-0.3 mg/mL and fungicidal effects at 0.15-0.6 mg/mL. The aqueous extract showed MICs of 0.15-2.5 mg/mL and MFCs at 0.60-5.0 mg/mL. The highest activity was observed for the ethyl acetate fraction, while the aqueous fraction showed the lowest antifungal effect. The most sensitive microfungi was *P. ochrochloron*, on the other hand, *A. fumigatus* was the most resistant to the tested fractions. The ethyl acetate fraction showed higher antifungal effect towards *P. ochrochloron* than both mycotics. All the tested fractions exhibited higher antifungal activity against *P. funiculosum* than ketoconazole. Chloroform and ethyl acetate fractions possessed higher effect on *P. verrucosum* var. *cyclopium* than ketoconazole. The Chloroform extract showed the same MIC but higher MFC for *A. niger* and *P. ochrochloron* than ketoconazole. In general, bacteria are more resistant than fungi (Soković et al., 2010). The

observed antibacterial activity from the chloroform fraction can be attributed to the presence of compounds; stigmasterol isolated from that fraction has a moderate antibacterial effect when compared to the reference drug (ciprofloxacin). This confirmed the antibacterial activity of stigmasterol against *Acetobacter* sp., *E. coli*, *S. aureus*, *Streptococcus* sp., and *P. aeruginosa* (Sileshi *et al.*, 2012); *β*-sitosterol also has a moderate antibacterial effect (Sileshi *et al*., 2012). Apigenin has a good antibacterial effect against several bacterial strains and the values are close to the antibacterial property of broad-spectrum, antibiotic tetracycline (Rui *et al.*, 2013). Also, only chloroform fraction possessed coumarins which can have influence in its higher antibacterial effect. It has been reported that some groups in coumarins skeleton result in increasing lipophilicity of the molecule (Stavri and Gibbons, 2005).

Table 1. Antibacterial activity of different fractions of *Cestrum nocturnum* aerial parts.

Bacteria	Chloroform MIC (mg/mL)* MBC (mg/mL)	Ethyl acetate MIC (mg/mL) MBC (mg/mL)	Aqueous MIC (mg/mL) MBC (mg/mL)	Streptomycin MIC (mg/mL) MBC (mg/mL)	Ampicillin MIC (mg/mL) MBC (mg/mL)
Bacillus	0.6±0.06	1.25±0.10	1.25±0.00	0.05±0.005	0.1±0.05
cereus	1.25±0.10	5.0±0.00	2.5±0.30	0.1±0.03	0.15±0.05
Micrococcus flavus	0.6±0.00	1.25±0.10	1.25±0.00	0.125±0.015	0.1±0.00
	2.5±0.30	5.0±0.60	2.5±0.00	0.25±0.003	0.15±0.06
Staphylococcus	0.6±0.06	2.5±0.30	1.25±0.10	0.25±0.003	0.1±0.05
aureus	2.5±0.00	5.0±0.30	2.5±0.30	0.50±0.00	0.15±0.00
Listeria	2.5±0.30	2.5±0.00	1.25±0.10	0.15±0.05	0.15±0.03
monocytogenes	5.0±0.60	5.0±0.00	5.0±0.60	0.30±0.03	0.30±0.01
Escherichia coli	0.6±0.00	1.25±0.10	1.25±0.10	0.10±0.03	0.30±0.05
	2.5±0.30	2.5±0.30	2.5±0.30	0.50±0.06	0.50±0.06
Pseudomonas	1.25±0.10	3.75±0.10	1.25±0.00	0.05±0.006	0.10±0.00
aeruginosa	2.5±0.30	7.5±0.00	2.5±0.30	0.10±0.0	0.20±0.05
Enterobacter	0.6±0.06	1.25±0.10	0.6±0.60	0.05±0.005	0.15±0.00
cloacae	2.5±0.30	5.0±0.60	1.25±0.00	0.10±0.06	0.20±0.06
Salmonella	1.25±0.00	1.25±0.00	1.25±0.00	0.05±0.006	0.15±0.03
typhimurium	2.5±0.30	5.0±0.30	2.5±0.30	0.10±0.03	0.20±0.00

* Minimum inhibitory concentration - MIC; Minimum bactericidal concentration - MBC.

Table 2. Antifungal activity of different fractions of *Cestrum nocturnum* aerial parts.

Fungi	Chloroform MIC (mg/mL)* MBC (mg/mL)	Ethyl acetate MIC (mg/mL) MBC (mg/mL)	Aqueous MIC (mg/mL) MBC (mg/mL)	Bifonazole MIC (mg/mL) MBC (mg/mL)	Ketoconazole MIC (mg/mL) MBC (mg/mL)
Aspergillus	0.6±0.06	0.3±0.00	2.5±0.00	0.15±0.03	0.20±0.03
fumigatus	1.25±0.10	0.6±0.06	5.0±0.60	0.20±0.06	0.50±0.05
Aspergillus	0.6±0.00	0.3±0.06	0.6±0.06	0.10±0.05	0.20±0.03
versicolor	1.25±0.00	0.6±0.00	2.5±0.30	0.20±0.03	0.50±0.05
Aspergillus	0.3±0.06	0.3±0.06	0.6±0.06	0.15±0.03	0.15±0.03
ochraceus	0.6±0.06	0.6±0.00	1.25±0.00	0.20±0.06	0.20±0.06
Aspergillus	0.2±0.03	0.2±0.03	0.6±0.06	0.15±0.03	0.20±0.06
niger	0.3±0.00	0.6±0.06	1.25±0.10	0.20±0.03	0.50±0.05
Penicillium	0.2±0.03	0.2±0.03	0.6±0.06	0.15±0.05	1.0±0.30
verrucosum	0.3±0.03	0.6±0.06	1.25±0.00	0.20±0.03	1.0±0.30
Penicillium	0.15±0.03	0.075±0.00	0.15±0.03	0.20±0.06	0.20±0.03
ochrochloron	0.3±0.06	0.15±0.03	0.6±0.06	0.25±0.05	0.50±0.06
Penicillium	0.3±0.00	0.3±0.06	0.15±0.00	0.20±0.03	2.5±0.30
funiculosum	0.6±0.00	0.6±0.00	0.6±0.06	0.25±0.05	3.5±0.50
Trichoderma	0.6±0.06	0.3±0.06	0.15±0.00	0.10±0.05	0.20±0.03
viride	1.25±0.00	0.6±0.00	0.6±0.06	0.20±0.06	0.30±0.06

* Minimum inhibitory concentration - MIC; Minimum fungicidal concentration - MFC.

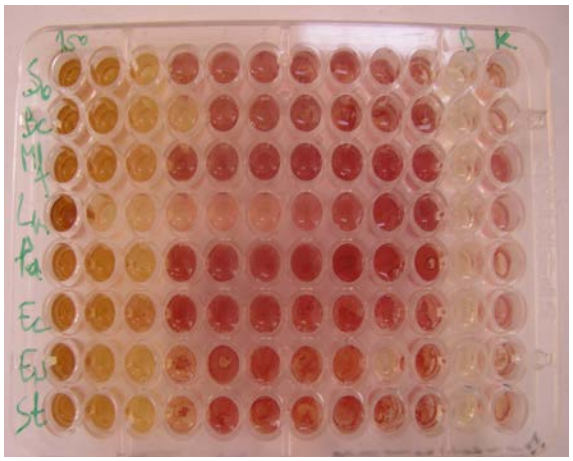

Figure 1. Antibacterial activity of the chloroform extract of *Cestrum nocturnum* aerial parts by microdilution method. Antibacterial effect of extract at different concentrations 0.6-2.5 mg/mL (MIC and MBC). Comparison between control-bacterial growth (K-red color) and treated samples (yellow color MIC and MBC) and treated samples with no activity (red color) in INT assay.

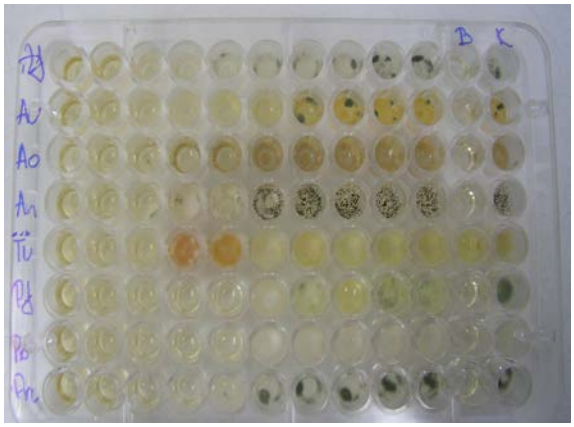

Figure 2. Antifungal activity of the chloroform extract of *Cestrum nocturnum* aerial parts by microdilution method. Control (K-fungal growth) and treated samples (MIC and MFC, 0.2-1.25 mg/mL–clean, transparent wells with no fungal growth, rows 1-3) and treated samples with fungal growth and no activity, rows 4-10.

Kaempferol isolated from ethyl acetate fraction showed a significant antimicrobial activity against all the tested organisms with MIC values between 16 and 63 µg/mL (Teffo *et al.*, 2010). Kaempferol di-sugars isolated from aqueous fraction showed a good antimicrobial activity against most of the studied microorganisms (Salwa *et al.*, 2012).

The three fractions revealed cytotoxicity against different human tumor cell lines (Table 3). Nevertheless, the chloroform fraction proved to be more active against NCI-H460 (lung carcinoma; GI_{50} 70.54 µg/mL), HCT-15 (colon carcinoma; GI_{50} 86.65 µg/mL) and HepG2 (hepatocellular carcinoma, GI_{50} 171.83 µg/mL) cell lines, while the ethyl acetate and the aqueous fractions were more efficient against HeLa (cervical carcinoma, GI_{50} 57.48 µg/mL) and MCF-7 (breast carcinoma, GI_{50} 55.28 µg/mL) cell lines, respectively. The ethyl acetate fraction (up to 400 µg/mL) was not toxic to non tumor liver cells; the other two fractions showed some hepatotoxicity, but at higher concentrations than the ones allowing anti-tumor effects. Ellipticine, a positive control, was used as a standard for comparison with the studied fractions.

The Cytotoxic activity observed for the chloroform fraction can be attributed to the presence of apigenin which showed a significant cytotoxic effect against different tumor cell lines (Rui *et al.*, 2013). The observed cytotoxic activity of ethyl acetate fraction can be attributed to the presence of the main flavonoid constituent, luteolin 7-*O*-glucoside which showed significant cytotoxicity (LD_{50} = 85 ±34 μg/mL) (Rilka *et al*., 2003), while the observed cytotoxicity of the aqueous fraction can be attributed to the presence of kaempferol di-sugars. Many reports showed that kaempferol glycosides cause cell death in a variety of cancer cells (Brusselmans *et al.*, 2005, Tomczyk *et al*., 2008).

The results of phytochemical analysis of the fractions of *C. nocturnum* aerial parts from the methanol 80 % extract are included in Table 4. Triterpenes, coumarins and flavonoids were detected in the chloroform fraction. Flavonoids, triterpenes and carbohydrates were detected in the ethyl acetate fraction, while tannins, flavonoids, saponins, carbohydrates and triterpenes were detected in the aqueous fraction. Isolation and purification of chloroform, ethyl acetate and aqueous fractions allowed the identification of ten bioactive compounds: β-sitosterol, stigmasterol, protocatechuic acid and apigenin from chloroform fraction; kaempferol 8-*O*-methyl ether, kaempferol, kaempferol 3-*O*-α-rhamnoside and luteolin 7-*O*-β-glucoside from ethyl acetate fraction; and kaempferol-disugars, kaempferol 3-*O*-β-glucoside-7-O-α-rhamnoside and kaempferol 3,7-di-*O*-α-rhamnoside from aqueous fraction (Figure 3). The chemical structures were determined by UV, ^{1}H-NMR, ^{13}C-NMR, and MS analyses.

Table 3. Cytotoxicity of different fractions of *Cestrum nocturnum* aerial parts in human tumor cell lines and in non-tumor liver primary culture.

	Chloroform (GI_{50}, µg/mL)*	Ethyl acetate (GI_{50}, µg/mL)	Aqueous (GI_{50}, µg/mL)	Ellipticine (GI_{50}, µg/mL)
HCT-15 (colon carcinoma)	86.65±5.86	223.00±4.03	98.11±8.48	1.91±0.06
HepG2 (hepatocellular carcinoma)	171.83±11.85	213.18±9,50	225.86±16.92	3.22±0.67
HeLa (cervical carcinoma)	62.49±4.36	57.48±5.37	61.34±3.59	1.14±0.21
MCF-7 (breast carcinoma)	66.22±5.70	194.22±5.73	55.28±4.87	0.91±0.04
NCI-H460 (lung carcinoma)	70.54±6.56	185.27±5.18	83.41±0.91	1.42±0.00
PLP2 (non-tumour liver cells)	323.44±9.67	>400	285.44±6.03	2.06±0.03

* GI_{50} values correspond to the sample concentration achieving 50% of growth inhibition in human tumour cell lines or in liver primary culture PLP2.

Table 4. Phytochemical analysis of different fractions of *Cestrum nocturnum* aerial parts.

Chemical Constituents	Chloroform	Ethyl acetate	Aqueous
Carbohydrates and/or glycosides	- *	+	+
Tannins			
Condensed tannins	-	-	+
Hydrolysable tannins	-	-	+
Alkaloids and/or nitrogenous bases	-	-	-
Flavonoids	+	+	+
Sterols and/or triterpenes	+	+	+
Saponins	-	-	+
Coumarins	+	-	-

* (-) denotes the absence of the constituents; (+) denotes the presence of the constituents.

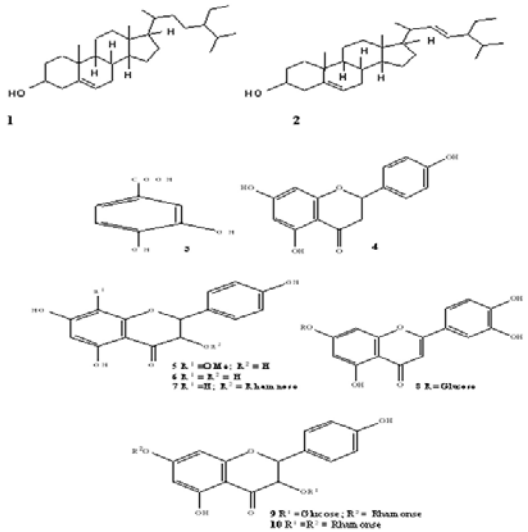

Figure 3. Chemical structure of the compounds isolated and identified in chloroform (1. *β*-sitosterol, 2. stigmasterol, 3. protocatechuic acid, 4. apigenin), ethyl acetate (5. kaempferol 8-*O*-methyl ether, 6. kaempferol, 7. kaempferol 3-*O*-α-rhamnoside, 8. luteolin 7-*O*-β-glucoside) and aqueous (9. kaempferol 3-*O*-β-glucoside-7-*O*-α-rhamnoside, 10. kaempferol 3,7-di-*O*-α-rhamnoside) fractions.

4. Conclusions

The present results proved that the chloroform fraction possessed the highest antibacterial activity due to the presence of coumarins and the presence of *β*-sitosterol, stigmasterol, protocatechuic acid and apigenin which could also contribute to the highest antibacterial activity of that fraction, and to the highest cytotoxicity against lung, colon and hepatocellular carcinoma cell lines. On the other hand, compounds such as kaempferol 8-*O*-methyl ether, kaempferol, kaempferol 3-*O*-α-rhamnoside and luteolin 7-*O*-*β*-glucoside could be related to the highest antifungal activity and cytotoxicity against cervical carcinoma cell line, displayed by the ethyl acetate fraction. Nevertheless, the different bioactivities exhibited by the fractions can also be due to the synergistic effect of different compounds present in the fractions or by the presence of other compounds that may be active even in small concentrations. The results obtained herein support the traditional medicinal use of *Cestrum nocturnum*, and provide grounds for further establishment of its use. Further studies are required to develop strategies for practical application.

Conflict of interest

There is no conflict of interest associated with the authors of this paper.

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