SUPPLEMENTARY MATERIAL

Chemical Composition and Biological Evaluation of the Volatile Constituents from the Aerial Parts of *Nephrolepis exaltata* (L.) and *Nephrolepis cordifolia* (L.) C. Presl Family Grown in Egypt

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Comparative investigation of hydro-distilled volatile constituents from aerial parts (A) of *Nephrolepis exaltata* (NE) and *Nephrolepis cordifolia* (NC) (F. Nephrolepidaceae) was carried out. Gas chromatography/mass spectrometry (GC/MS) revealed that oils differ in composition and percentages of components. Oxygenated compounds were dominant in NEA and NCA, being 72.2% and 54.3%, respectively. 2, 4-Hexadien-1-ol (16.1%), nonanal (14.4%), β -Ionone (6.7%) and thymol (2.7%) were predominant in NEA. β -Ionone (8.0%); eugenol (7.2%) and anethol (4.6%) were the main constituents in NCA. Volatile samples were screened for their antibacterial and antifungal activities using agar diffusion method (ADM) and minimum inhibitory concentrations (MIC). The cytotoxic activity was evaluated using viability assay in breast (MCF-7), colon (HCT-116) and lung carcinoma (A-549) cells by the MTT assay. The results revealed that NEA oil exhibited potential antimicrobial activity against most of the tested organisms and showed promising cytotoxicity.

Experimental

Plant material

Nephrolepis exaltata (L.) and *Nephrolepis cordifolia* (L.) Presl (F. Nephrolepidaceae) aerial parts were collected in June 2013 from experimental station, faculty of pharmacy, Cairo University, Egypt. The plant samples were identified by Miss Mervat Abdelrehim, Taxonomist, El-Orman Botanical garden, Giza, Egypt. A voucher specimen (no. 929 and 930) was deposited in the herbarium of Misr International University.

Bacterial and fungal strains

Gram-negative bacteria: *Staphylococcus aureus* (RCMB 010027), *Staphylococcus pneumoniae* (RCMB 010010) and *Enterococcus faecalis* (RCMB 010068); Gram-positive bacteria: *Salmonella typhimurium* (RCMB 0010072), *Proteus vulgaris* (RCMB 010085), *Klebsiella pneumoniae* (RCMB 0010093), *Shigella flexneri* (RCMB 0100542), *Pseudomonas aeruginosa* (RCMB 010043), *Escherichia coli* (RCMB 010056) and fungi: *Microsporum gypseum* (RCMB 06225), *Tricophyton rubrum* (RCMB 09358) and *Tricopyton metagrophytes* (RCMB 0925) were offered by The Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

Cell lines

Human Breast cancer (MCF-7), colon carcinoma (HCT-116) and lung carcinoma (A-549) cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50µg/ml gentamycin. The cells were maintained at 37°C in a humidified atmosphere with 5% CO₂ and were sub-cultured two to three times a week.

Extraction of volatile constituents and GC/MS analysis

Five hundred grams of the fresh aerial parts of NE and NC, each separately, was subjected to hydro-distillation in a Clevenger's apparatus for 5 hours according to Egyptian pharmacopoeia 2005. The oils were stored in sealed glass vials and maintained in a refrigerator till analysis.

Gas Chromatography-Mass Spectrometry

The volatile oils were subjected to GC/MS analysis on a Agilent 6890 gas chromatograph equipped with an Agilent mass spectrometric detector, with a direct capillary interface and fused silica capillary column PAS -5 ms (30 m x 0.32 mm x 0.25 µm film thickness) (Formacek 1982). Individual components were identified by matching their mass spectra with those of the spectrometer data base using Wiley and NIST computer library as well as by comparison of the fragmentation pattern with those reported literature (Adams 2009). The identified components, retention times and Kovat's indices are presented in Table 1.

Antimicrobial activity

Each volatile oil under investigation in 100 µl aliquots was dissolved in 1 ml dimethyl sulfoxide (DMSO) and individually tested against a panel of Gram-positive and Gram-negative bacteria and fungi. Antimicrobial activity was carried out using the agar well diffusion method (Scott 1989). Negative controls were prepared using DMSO employed for dissolving the tested compound. Ampicillin (50µg/ml), Gentamicin (50µg/ml) and Amphotericin B (50µg/ml) were used as standards for Gram-positive bacteria, Gram-negative bacteria and fungi, respectively. Antimicrobial activity was expressed as inhibition diameter zones in millimeters (mm). The experiment was carried out in triplicate and the average zone of inhibition was calculated. The data was expressed as mean ± SD (Table S1 and S2).

Determination of MIC

The MIC was determined by the micro-broth kinetic assay. The percentage of growth at each sample concentration was calculated with the following equation: % growth [(OD600 of wells containing the sample/OD600 of the sample-free well) x 100] after subtraction of background ODs (ODs of microorganism-free wells) (Kaya et al., 2009) (Table S1 and S2).

Evaluation of the antitumor activity

The antitumor activity was determined by MTT assay based on cell viability adopting the method reported by (Mosmann 1983; Gangadevi & Muthumary 2007). The number of the surviving cells was determined by staining the cells with crystal violet followed by cell lysing using 33% glacial acetic acid and read the absorbance at 590nm using ELISA reader (SunRise, TECAN, mc, USA) after well mixing. The

absorbance values from untreated cells were considered as 100% proliferation. The number of viable cells was determined using ELISA reader as previously mentioned and the percentage of viability was calculated as $[1 - (OD_t/OD_c)] \times 100\%$. OD_t is the mean optical density of wells treated with the tested sample and OD_c is the mean optical density of untreated cells. The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots (Table S3).

References

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Table S1. Antimicrobial activity and minimum inhibitory concentration (MIC) of volatile constituents of aerial parts of NE and NC cultivated in Egypt

Tested microorganism	NEA		NCA		Standard	
	^a Zone of inhibition	^b MIC	^a Zone of inhibition	^b MIC	^a Zone of inhibition	^b MIC
Gram Positive Bacteria					<i>Ampicillin</i>	
<i>Staphylococcus aureus</i> (RCMM 010027)	21.4±0.3	0.98*	21.4±0.3	0.98*	28.9±0.1	0.015
<i>Streptococcus pneumoniae</i> (RCMM 010010)	20.2±0.4	1.95*	20.2±0.4	1.95*	25.4±0.2	0.06
<i>Enterococcus faecalis</i> (RCMB 010068)	19.9±0.4	1.95*	19.9±0.4	1.95*	26.4±0.3	0.03
Gram negative Bacteria					<i>Gentamycin</i>	
<i>Salmonella typhimurium</i> (RCMB0010072)	19.4±0.1	3.9*	NA	NA	19.9±0.2	1.95
<i>Proteus vulgaris</i> (RCMB 010085)	17.7±0.4	7.81*	NA	NA	23.4±0.3	0.24
<i>Klebsiella pneumonia</i> (RCMB 0010093)	22.9±0.3	0.24*	18.4±0.6	3.9*	26.3±0.2	0.03
<i>Shigella flexneri</i> (RCMB 0100542)	22.4±0.3	0.49*	16.7±0.6	15.63	24.8±0.2	0.12
<i>Pseudomonas aeruginosa</i> (RCMB 010043)	14.2±0.4	125	NA	NA	17.3±0.1	15.63
<i>Escherichia coli</i> (RCMB 010056)	19.7±0.4	1.95*	17.6±0.2	15.63	25.3±0.2	0.063

^aMean zone of inhibition in mm ± Standard deviation (SD) beyond well diameter (6mm) where n=3 using (100µL/ml) concentration of tested samples. ^bMIC minimum inhibitory concentration (µg/ml) of tested samples against tested microorganisms. NA: No activity, RCMB: Regional Centre for Mycology and Biotechnology.

*sensitive microorganism

Table S2. Antifungal activity and minimum inhibitory concentration of volatile constituents of volatile constituents of aerial parts of NE and NC cultivated in Egypt

Tested microorganism	Minimum inhibitory concentration(µg/ml)		
	NEA	NCA	<i>Amphoterecin B</i>
<i>Microsporum gypseum</i> (RCMB 06225)	0.24*	0.98*	0.12
<i>Tricophyton rubrum</i> (RCMB 09358)	0.98*	3.90*	0.49
<i>Tricopyton metagrophytes</i> (RCMB 0925)	1.95*	07.81	0.06

Sample of 100µl was tested, RCMB: Regional Centre for Mycology and Biotechnology. Organisms are considered susceptible to amphotericin B, when the MIC is ≤1.0 µg/ml, intermediate when MIC is 2.0 µg/ml, and resistant when the MIC is ≥ 4.0 µg/ml. *sensitive microorganism

Table S3. Cytotoxicity of volatile constituents of volatile constituents of aerial parts of NE and NC cultivated in Egypt

Sample	HCT-116		MCF7		A-549	
	Viability %	IC ₅₀	Viability %	IC ₅₀	Viability %	IC ₅₀
NEA	31.47±3.9	89.4	19.64±1.4	44	16.75±0.9	43.5
NCA	17.26±0.9	70.9	26.51±2.8	47.9	21.13±0.4	46.2
<i>Doxrubicin</i>	6.82	0.47	3.24	0.426	4.98	0.94

Sample conc. 100 µg/ml; Doxrubicin conc. 50 µg/ml; Viability % ± SD; IC₅₀ expressed in µg/ml; limit of activity defined as IC₅₀ < 50 µg/ml.