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CHEMICAL COMPOSITION, ANTIMICROBIAL AND CYTOTOXIC ACTIVITIES OF VOLATILE CONSTITUENTS FROM THE SUBTERRANEAN ORGANS OF *NEPHROLEPIS CORDIFOLIA* (L.) C. PRESL AND *NEPHROLEPIS EXALTATA* (L.) FAMILY NEPHROLEPIDACEAE GROWN IN EGYPT

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ABSTRACT

Investigation of the chemical composition of the essential oils from subterranean organs (S) of *Nephrolepis cordifolia* (NC) and *Nephrolepis exaltata* (NE) (F. Lomariopsidaceae) grown in Egypt was carried out. Gas Chromatography/mass Spectrometry (GC/MS) analysis of the hydro-distilled NCS and NES oils revealed that oxygenated compounds were abundant, being 55.35% and 33.85%, respectively. Ethyl palmitate (8%), ethyl linolenate (6.33%), β-ionone (5.99%), phytol (3.93%) diterpene alcohol and α-cadinol (3.3%) were the major identified constituents in NCS. Linalool (8.23%), thymol (4.47%), methyl palmitate (2.75%), α-cadinol (2.04%), geraniol (1.66%) and eugenol (1.53%) were the majors in NES. The volatile samples were screened for their antimicrobial activities using the agar diffusion technique and the minimum inhibitory concentrations (MIC). The essential oils exhibited potential antibacterial and antifungal activities against most of the tested microorganisms, with diameters of inhibition zones ranging from 17.1 ± 0.42 to 19.2 ± 0.29 mm and MIC values from 3.9 to15.63µg/ml. The cytotoxic activity was determined by MTT assay based on cell viability in breast (MCF-7), colon (HCT-116) and lung (A-549) carcinoma cells. The study revealed remarkable cytotoxicity of NCS oil. The presence of phytol (3.93%), α-Cadinol (3.30%), α-Ionone (2.36%) and β-Ionone (5.99%) significantly contributed to the potent cytotoxic activities of NCS oil.

Keywords: *Nephrolepis cordifolia*, *Nephrolepis exaltata*, volatile constituents, antibacterial, antifungal, cytotoxicity

INTRODUCTION

In recent years, a large number of essential oils and their constituents have been investigated for their antimicrobial properties against bacteria and fungi. They have a wide range of applications and can be used for a variety of purposes, including food preservation, natural therapeutics, cosmetics and treatment of skin conditions (Grigore *et al*., 2012). The resistance which certain microorganisms have developed against antibiotics initiated investigations of essentials oils against a wide range of bacterial and fungal species (Alim *et al*., 2009). In addition, the diverse therapeutic potential of essential oils has drawn the attention of researchers to test them for anticancer activity, taking advantage of the fact that their mechanism of action is dissimilar to that of the classic cytotoxic chemotherapeutic agents (Edris, 2007). *Nephrolepis cordifolia* and *Nephrolepis exaltata* are terrestrial ferns with short rhizome and small tubers belonging to family, Nephrolepidaceae. They grow in subtropical and tropical regions and are commonly cultivated as ornamental ferns. They spread aggressively throughout by windblown spores or by accidental movement of tubers and rhizomes. Juice of root tubers of NC is taken to treat fever, cough and hematuria. NE is known to be non-toxic and is considered as natural air purifier (Kobayashi *et al*., 2007). Rani *et al*. (2010) screened the chemical composition of the aqueous and non-aqueous extracts of subterranean organs of *Nephrolepis cordifolia* grown in India and evaluated their antibacterial and antifungal activities. No information is available about the essential oils of NCS and NES grown in Egypt. Therefore, the objective of this study was to carry out a comparative investigation on the chemical composition of NCS and NES by GC/MS analysis and to assess their *in vitro* antibacterial, antifungal and cytrotoxic activities.

MATERIALS AND METHODS

Plant material

Nephrolepis cordifolia (L.) Presl and *Nephrolepis exaltata* (L.) Schott (Family Nephrolepidaceae) subterranean organs including roots and root tubers were

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collected on June 2013 from experimental station, faculty of pharmacy, Cairo University, Giza, Egypt. The plant samples were kindly identified by Miss Mervat Abdelrehim, Taxonomist, El-Orman Botanical garden, Giza, Egypt. A voucher specimen (no. 930 and 929) has been 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 pneumonia* (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 kindly offered by The Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

Cell line

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% CO_2 and were sub-cultured two to three times a week.

Preparation of volatile oils

Five hundred grams of NCS and NES, each separately, was subjected to hydro-distillation in a Clevenger's apparatus for 5 hours according to Egyptian pharmacopoeia 2005. The volatile distillates were stored in sealed dark glass containers and maintained in a refrigerator for analysis.

Gas Chromatography-Mass Spectrometry

Analysis of the volatile oils were carried out on a an Agilent 6890 gas chromatograph equipped with 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). Helium was used as carrier gas with flow rate 1ml/min., pulsed in splitless mode. Detector was operated in EI ionization mode of 70 eV; scanning was done from *m/z* 50 to 500. The ion source temperature was 230°C and the quadrupole temperature was 150°C. The electron multiplier voltage was maintained at 1250V. Temperature program was started at 60°C then elevated to 280°C at rate of 8°C/mm hold at 280°C. The detector and injector temperature were 280 and 250°C, respectively (Formacek, 1982). Components were identified by matching their mass spectra with Wiley and NIST data base library as well as by comparison with literature (Adams, 2009). The identified components, retention times and Kovat's indices are presented in table 1.

Antimicrobial activity

Each volatile oil in 100µl aliquots was separately tested against a panel of Gram-positive, Gram-negative bacteria and fungi using the agar well diffusion method described

Table 1. GC/MS analysis of volatile constituents of *Nephrolepis cordifolia* and *Nephrolepis exaltata* subterranean organs cultivated in Egypt.

No.	Identified Compound	Area Percentage			
		RT	KI	NCS	NES
1	2,4-Hexadien-1-ol	4.30	916	-	2.63
2	2,3-dimethyl-undec-1-en-3-ol	4.94	945	-	10.17
3	1-ethyl-2-methyl benzene	5.95	964	2.01	-
4	Pseudocumene	6.55	1023	3.19	-
5	Benzyl alcohol	7.39	1026	0.49	0.58
6	*p*-Mentha-1,5,8-triene	7.78	1090	5.40	1.73
7	*p*-cymene	8.31	1024	1.98	1.39
8	Linalool	8.57	1096	-	8.23
9	Durene	8.98	1114	1.27	2.63
10	Pentyl cyclohexadiene	9.58	1156	3.19	2.12
11	2-nonenal	9.70	1097	-	1.61
12	1,2,3,4-tetrahydro naphthalene	9.85	1164	-	1.15
13	Azulene	10.25	1298	2.99	-
14	Dodecane	10.39	1200	-	6.79
15	2,6-Dimethyl undecane	10.64	1215	-	0.61

Continue…

Table 1 continued...

No.	Identified Compound	Area Percentage			
		RT	KI	NCS	NES
16	Geraniol	11.43	1279	-	1.66
17	1,2,3,4-Tetrahydro-5-methyl naphthalene	11.67	-	-	1.4
18	Thymol	12.16	1289	0.42	4.47
19	2-methyl naphthalene	12.21	1281	1.88	-
20	1- methyl naphthalene	12.51	1298	0.80	-
21	Eugenol	13.24	1356	-	1.53
22	3-methyl tridecane	13.35	1372	-	0.64
23	Tetradecane	13.84	1400	-	4.66
24	1,5-dimethyl naphthalene	14.32	1446	2.25	-
25	3-methyl tetradecane	14.94	1462	-	1.42
26	β- Ionone	15.36	1488	5.99	-
27	Pentadecane	15.41	1500	-	4.22
28	Dibenzofuran	15.88	1512	0.77	-
29	Methyl 7,9-tridecadienyl ether	16.23	-	-	1.47
30	3-methyl pentadecane	16.46	-	-	1.90
31	Hexadecane	16.88	1600	3.14	3.71
32	α- Cadinol	17.66	1652	3.30	2.04
33	Heptadecane	18.31	1700	-	4.97
34	3-methyl heptadecane	18.91	-	-	2.68
35	Octadecane	19.62	1800	0.55	1.06
36	2,6,11,15 tetramethyl hexadecane	20.26	1810	-	1.09
37	Dibutyl phthalate	21.27	1922	1.85	1.33
38	Methyl palmitate	21.80	1927	1.54	2.75
39	Ethyl palmitate	22.09	1993	8.00	-
40	α-cadrene epoxide	22.78	-	1.48	-
41	Heneicosane	23.31	2100	-	0.55
42	Phytol isomer	23.51	2100	3.93	-
43	Methyl oleate	23.64	-	0.54	-
44	Ethyl linolate	24.06	2092	0.97	-
45	Ethyl linolenate	24.13	2108	6.33	-
46	Ethyl stearate	24.39	2194	3.19	-
47	Docosane	24.44	2200	-	0.42
48	Benzyl butyl phthalate	26.20	-	1.34	-
49	Tetracosane	26.53	2400	1.81	-
50	Pentacosane	27.53	2500	0.53	-
Sum				71.13	83.61
% of oxygenated compounds				55.35	33.85
% of hydrocarbons				44.45	66.15

by Scott (1989). Negative controls were prepared using DMSO for dissolving the samples. Fifty µg/l of each of Ampicillin, Gentamicin and Amphotericin B 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. MIC of tested samples was determined by the micro-broth kinetic assay, the percentage of growth was calculated according to the method reported by Kaya *et al*. (2009).

Evaluation of the antitumor activity

The antitumor activity was determined by MTT assay based on tumor cell viability adopting the method reported by Mosmann (1983) and Gangadevi and Muthumary (2007). A positive control containing a Doxrobicin drug was also tested as reference drug for comparison. Untreated cells was made in absence of tested sample and kept as control. Six wells were used for each concentration of the test sample. The number of the surviving cells was determined and the viability percentage was calculated. The 50% inhibitory concentration (IC_{50}) was appraised from graphical plots.

RESULTS AND DISCUSSION

Chemical composition of the essential oil

Hydro-distillation of NCS and NES yielded 1.0 and 0.65 v/w% of pale yellow oils with a pleasant aroma. Analysis of the volatile constituents (Table1) revealed that the oils differ in composition and percentages of components. The total number of identified constituents was 29 and 32 in NCS and NES oil samples; representing 71.13% and 83.61% of the total oil composition. Oxygenated compounds were found to be 55.35% in NCS and 33.85% in NES oils.

Components of the NCA and NES essential oils were identified as alcohols (10.85, 18.11%), phenols (0.6, 7.17%), ketones (8.42, 0.0%), aldehydes (0.0, 1.93%) and esters (33.40, 4.88%). The results revealed that the major identified oxygenated compounds in NCS were ethyl palmitate (8%), ethyl linolenate (6.33%), β-ionone (5.99%), phytol (3.93%) and α-cadinol (3.3%). Linalool (8.23%), thymol (4.47%), methyl palmitate (2.75%), α-cadinol (2.04%), geraniol (1.66%) and eugenol (1.53%) were the majors in NES.

Antimicrobial activity

Most of the tested bacterial organisms showed sensitivity to NCS and NES oils compared to standard antibiotics (Table 2). MIC values of NCS oil against Gram-positive bacteria such as *Streptococcus aureus, Staphylococcus pneumoniae* and *Enterococcus faecalis* were 3.9, 3.9 and 15.63µg/ml, respectively. MIC values indicated sensitivity of these organisms to NCS oil compared to Ampecillin. Although *Enterococcus faecalis* is known to be an intrinsically resistant bacterium to several commonly used antibiotics, our MIC values of NCS oil was 15.63µg/ml indicating promising activity. However, NES showed good activity against only *Staphylococcus aureus* with MIC 7.81µg/ml. Both examined oils showed much less activity against Gram-negative bacteria. The MIC values of NCS oil against *Klebsiella pneumoniae and Escherichia coli* were 15.63 and 7.81µg/ml, respectively. NES oil showed lower MIC value against *Klebsiella pneumoniae* and *Escherichia coli* with MIC 0.98µg/ml and 3.90µg/ml, respectively. According to the hydrophobic nature of NES, less activity was expected against Gram-negative bacteria. Contradicting results of higher activity could be attributed to alcohols as linalool and the phenolic structures of the oil components as

Table 2. Antimicrobial activity and minimum inhibitory concentration (MIC) of volatile constituents of NCS and NCS cultivated in Egypt

Tested microorganism	NCS		NES		Standard	
	[a] Zone of inhibition	[b] MIC	[a] Zone of inhibition	[b] MIC	[a] Zone of inhibition	[b] MIC
Gram positive Bacteria					*Ampicillin*	
Staphylococcus aureus (RCMM 010027)	18.6±0.21	3.9*	17.8±0.33	7.81*	28.9±0.14	0.015
Streptococcus pneumoniae (RCMM 010010)	19.2±0.29	3.9*	15.3±0.12	62.5	25.4±0.18	0.06
Enterococcus faecalis (RCMB 010068)	17.1±0.42	15.63*	16.3±0.39	31.25	26.4±0.34	0.03
Gram negative Bacteria					*Gentamycin*	
Salmonella typhimurium (RCMB0010072)	15.1±0.51	62.5	15.9±0.29	31.25	19.9±0.18	1.95
Proteus vulgaris (RCMB 010085)	NA	NA	11.9±0.33	125	23.4±0.30	0.24
Klebsiella pneumonia (RCMB 0010093)	17.4±0.39	15.63*	20.6±0.19	0.98*	26.3±0.15	0.03
Shigella flexneri (RCMB 0100542)	15.8±0.28	31.25	16.3±0.17	31.25	24.8±0.24	0.12
Pseudomonas aeruginosa (RCMB 010043)	NA	NA	NA	NA	17.3±0.12	15.63
Escherichia coli (RCMB 010056)	17.6±0.13	7.81*	18.5±0.17	3.90*	25.3±0.18	0.06

[a]Mean zone of inhibition in mm ± Standard deviation (SD) beyond well diameter (6mm) where n=3 using (125µL/ml) concentration of tested samples. [b]MIC minimum inhibitory concentration (µg/ml) of tested samples against tested microorganisms. NA: No activity, RCMB: Regional Centre for Mycology and Biotechnology. *Sensitive microorganism

Table 3. Antifungal activity and MIC of volatile constituents of NCS and NES

Tested microorganism	Minimum inhibitory concentration (µg/ml)		
	NCS	NES	*Amphoterecin B*
Microsporum gypseum (RCMB 06225)	3.9*	7.81	0.12
Tricophyton rubrum (RCMB 09358)	31.25	62.5	0.49
Tricopyton metagrophytes (RCMB 0925)	7.81	3.9	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 4. Cytotoxicity of volatile constituents of NCS and NES cultivated in Egypt

Sample	HCT-116		MCF7		A-549	
	Viability %	IC_{50}	Viability %	IC_{50}	Viability %	IC_{50}
NC S	13.93±0.46	40.8	9.14±0.34	37.6	7.86±0.23`	23.6
NE S	63.59±2.96	>200	17.86±2.64	43.2	13.97±2.04	24.3
Doxorubicin	6.82	0.47	3.24	0.426	4.98	0.94

Sample conc. 100 µg/ml; Doxorubicin conc. 50 µg/ml; Viability % ± SD; IC_{50} expressed in µg/ml; limit of activity defined as IC_{50}< 50 µg/ml.

thymol and eugenol as well as the possible synergistic interaction between components (Dorman & Deans 2000). Table 3 shows the MIC values of the oils against dermatophytes in comparison to standard Amphoterecin B. NCS showed potent activity against *Microsporum gypseum* with MIC value 3.9µg/ml while NES oil showed good activity against *Tricopyton metagrophytes* with MIC value 3.9µg/ml. The antimicrobial property is due to the capacity of various constituents to defeat infectious microorganisms and to inhibit their proliferation (Istudor, 2001).

Cytotoxic activity

The viability percentages and the IC_{50} values of the NCS and NES oils on human cancer cell lines colon (HCT-116), breast (MCF7) and lung (A-549) are shown in table 4. NCS oil revealed potent activity on the lung, breast and colon cell lines with IC_{50} of 23.6, 37.6 and 40.6µg/ml. This may be attributed to the presence of phytol (3.93%) diterpene alcohol, α-Cadinol (3.30%) and β- Ionone (5.99%) which has been reported with cytotoxic activities (Dasgupta and Humphrey, 1998; Sylvestre *et al*., 2007; Sua *et al*., 2013). NES oil was active against lung and breast carcinoma with IC_{50} of 24.3 and 43.2g/ml.

CONCLUSION

In the present work we demonstrated for the first time, the chemical composition, antibacterial and antifungal properties as well as the cytotoxic activity. The results indicated that NCS and NES oils exhibited antimicrobial activity against Gram-positive and Gram-negative bacteria. NCS oil showed potent activity against breast, colon and lung carcinoma cell lines. Additional *in vivo* studies and clinical trials will be needed to justify and further evaluate the potential of these oils as antimicrobial and cytotoxic agents.

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REFERENCES

Adams, RP. 2009. Identification of essential oils by gas chromatography/mass spectroscopy. (4th edi.). Carol Stream, USA. Allured Publication Corporation.

Alim, A., Goze, I., Cetin, A., Atas, A, D., Vural, N. and Donmez, E. 2009. Antimicrobial activity of the essential oil of *Cyclotrichium niveum* (Boiss.) Manden. Et Scheng. Afr. J. Microbiol. Res. 3:422-425.

Dasgupta, A. and Humphrey, PE. 1998. Gas chromatographic-mass spectrometric identification and quantitation of benzyl alcohol in serum after derivatization with perfluorooctanoyl chloride: A new derivative. J Chromatogr B Biomed Sci App. 708:299-303.

Dorman, HJ. and Deans, SG. 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J Appl Microbiol. 88:308-316.

Edris, AE. 2007. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: A review. Phytother Res. 21:308-323.

Egyptian Pharmacopoeia. 2005. General Organization for Government Printing Office. Cairo.

Formacek, V. and Kubeczka, KH. 1982. Essential oils analysis by capillary GC and C^{13} NMR spectroscopy. John Wiley & Sons. New York and London.

Gangadevi, V. and Muthumary, J. 2007. Preliminary studies on cytotoxic effect of fungal taxol on cancer cell lines. African Journal of Biotechnology. 6:1382-13 86.

Grigore, A., Colceru-Mihul, S., Paraschiv, I. Nita, S., Christof, R., Iuksel, R., Ichim, M. and Maria, I. 2012. Chemical analysis and antimicrobial activity of indigenous medicinal species volatile oils. Rom. Biotech. Lett. 17:7620-7627.

Istudor, V. 2001. Farmacognozie, fitochimie, fitoterapie. (vol. II). 27.

Kaya, EG., Ozbilge , H. and Albayrak, S. 2009. Determination of the effect of gentamicin against *staphylococcus aureus* by using for the determination of MIC of tested samples by the microbroth kinetic assay, microbroth kinetic system. Ankem Derg. 23:110-114.

Kobayashi, KD., Kaufman A., Griffis, J. and McConnell, J. 2007. Using Houseplants to Clean Indoor Air. Ornamentals and Flowers. 1-7.

Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods. 65:55-63.

Rani, D., Khare, PB. and Dantu, PK. 2010. *In Vitro* antibacterial and antifungal properties of aqueous and non-Aqueous frond extracts of *Psilotum nudum*, *Nephrolepis biserrata* and *Nephrolepis cordifolia.* Indian J Pharm Sci. 72:818-822.

Scott, AC. 1989. Laboratory control of antimicrobial therapy. In: Mackie and McCartney Practical medical microbiology. Eds. Collee, JG., Duguid, JP., Fraser, AG. and Marmion, BP. (vol. 2, 13^{th} edi.). United Kingdom-Edinburgh: Churchill Livingstone, 161-181.

Sule, AM., Thanni, LOA., Sule Sua, Y., Hsub, K., Wangb, EI. and Hob, C. 2013. Composition and *in vitro* anticancer activities of the leaf essential Oil of *Neolitsea variabillima* from Taiwan. Nat Prod Commun. 8:531-532.

Sylvestre, M., Pichette, A., Lavoie, S., Longtin, A. and Legault, J. 2007. Composition and cytotoxic activity of the leaf essential oil of *Comptonia peregrina* (L.) Coulter. Phytother Res. 21:536-40.

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