SHORT COMMUNICATION

Chemical composition and biological evaluation of the volatile constituents from the aerial parts of *Nephrolepis exaltata* (L.) and *Nephrolepis cordifolia* (L.) C. Presl grown in Egypt

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The essential oil from the aerial parts of *Nephrolepis exaltata* and *Nephrolepis cordifolia* obtained by hydro-distillation were analyzed by gas chromatography/ mass spectrometry. The essential oils exhibited potential antibacterial and antifungal activities against a majority of the selected microorganisms. NEA oil showed promising cytotoxicity in breast, colon and lung carcinoma cells. The results presented indicate that NEA oil could be useful alternative for the treatment of dermatophytosis.

Comparative investigation of hydro-distilled volatile constituents from aerial parts (A) of *Nephrolepis exaltata* (NE) and *Nephrolepis cordifolia* (NC) (Family Nephrolepidaceae) was carried out. Gas chromatography/mass spectrometry revealed that oils differ in composition and percentages of components. Oxygenated compounds were dominant in NEA and NCA. 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 and minimum inhibitory concentrations. 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.

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

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1. Introduction

_Nephrolepis exaltata_ (NE) and _Nephrolepis cordifolia_ (NC) (Family Nephrolepidaceae) are terrestrial ferns and commonly cultivated as ornamental plants. NC is used to cure renal, liver and skin disorder (Dhiman 1998). NE acts as a natural air humidifier and purifier (Kobayashi et al. 2007). Rani et al. (2010) reported the phytochemical screening as well as the antibacterial and antifungal effects of ethanol extract of aerial parts of NC grown in India. To evaluate the possible use of the essential oils of NCA and NEA grown in Egypt for topical applications for skin infections, gas chromatography/mass spectrometry (GC/MS) was performed on both extracts to identify their antibacterial, antifungal and cytotoxic activities.

2. Results and discussion

2.1. Chemical composition of the essential oil

Hydro-distillation of NEA and NCA yielded 0.63% and 0.65% of pale yellow oils with a pleasant aroma, respectively. GC/MS analysis revealed that the oils differ in composition and percentages of components (Table S1). The total number of identified constituents was 23 and 31 in NEA and NCA oils, representing 91.80% and 78.8% of the total composition, respectively. Oxygenated compounds were dominant in NEA and NCA oils, being 72.2% and 54.3 %, respectively. They were identified as aldehydes (26.4% and 18.7%), alcohols (33.7% and 2.4%), ketones (7.2% and 13.2%) and alcohols (2.9% and 9.1%). The results revealed that 2,4-Hexadien-1-ol (16.1%), nonanal (14.4%), nonenal (9.3%), β-ionone (6.7%), thymol (2.7%) and nonanoic acid (1.1%) were predominant in NEA. Nonanal (10.6%), β-ionone (8.0%), eugenol (7.2%) and anethol (4.6%) were the major constituents in NCA (Table 1).

2.2. Antimicrobial activity

Volatile oils were screened for their antimicrobial activities using agar diffusion method and minimum inhibitory concentration (MIC) (Table S1). The MIC values of NEA oil against Gram-negative bacterial strains _Salmonella typhimurium_, _Klebsiella pneumoniae_, _Shigella flexneri_ and _Escherichia coli_ were 3.9, 0.24, 0.49 and 1.95 μg/mL, respectively, whereas MIC against _Proteus vulgaris_ was 7.81 μg/mL. MIC values indicated sensitivity of these organisms to NEA oil compared to gentamycin. In spite of the hydrophobic nature of the oil, the highest activity was observed against _K. pneumoniae_ and _S. flexneri_. NEA showed a potent activity against a majority of the selected organisms except _Pseudomonas aeruginosa_ with an MIC value of 125 μg/mL indicating resistance of the organism to the oil. MIC values against _Staphylococcus aureus_, _Streptococcus pneumoniae_ and _Enterococcus faecalis_ were 0.98, 1.95 and 1.95 μg/mL, respectively. Our study revealed less activity of NEA oil on Gram-positive bacteria as compared to Ampicillin. Although _E. faecalis_ is known to be an intrinsically resistant Gram-positive bacterium, MIC values of NEA oil showed promising activity. Most of the antimicrobial activities could be associated with the presence of thymol, nonenal, nonanal, _p_-cymene, α-ionone, β-ionone as well as anethol which are well-known substances with pronounced antimicrobial properties (Chatterjee and Kundu 1975; Mikhlin et al. 1983; Chao et al. 2000; Edris 2007). The antibacterial activity of NCA showed remarkable activity against _K. pneumoniae_ with MIC 3.9 μg/mL. The oils were also screened for their antifungal activity (Table S2) in comparison to standard Amphoterecin B. NEA oil showed potent activity against _Microsporum gypseum_, _Tricophyton rubrum_ and _Tricophyton metagrophytes_ with MIC values 0.24, 0.98 and 1.95 μg/mL, respectively. However, NCA showed good activity against _M. gypseum_ and _T. rubrum_ with MIC values of 0.98 and 3.9 μg/mL, respectively. It is evident that there is a relation between the strong antimicrobial activity and the high percentage of oxygenated compounds. This is in agreement with the finding of Kalemba and Kunicka (2003)
as well as that of Dorman and Deans (2000). The skin has an extremely diverse ecology of organisms that may produce infections. Empirical therapy for skin infections must include coverage of the commonly encountered pathogen. In addition, antimicrobial therapy could be complicated due to emergence of antibiotic resistance among different pathogens. Accordingly,

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

| No. | Identified compound                        | Area percentage | | | |
|-----|------------------------------------------|----------------|---|---|---|
|     |                                          | RT  | KI  | NCA | NEA    | |
| 1   | 2,4-Hexadien-1-ol                        | 4.30| 916 | –   | 16.09  | |
| 2   | 2,3-Dimethyl-undec-1-en-3-ol              | 4.94| 945 | –   | 4.49   | |
| 3   | 1-Ethyl-2-methyl benzene                  | 5.95| 964 | 0.44| –       | |
| 4   | 1-Octen-3-ol                             | 6.21| 976 | 1.51| 14.34  | |
| 5   | Pseudocumene                             | 6.55| 1023| 2.63| –       | |
| 6   | Benzyl alcohol                           | 7.39| 1026| –   | 0.49   | |
| 7   | *p*-Mentha-1,5,8-triene                   | 7.78| 1090| 1.89| 0.76   | |
| 8   | *p*-Cymene                               | 8.31| 1024| 1.69| –       | |
| 9   | Nonanal                                  | 8.69| 1100| 10.56| 14.39 | |
| 10  | Durene                                   | 8.98| 1114| 3.55| –       | |
| 11  | 2-Nonenal                                | 9.70| 1162| –   | 9.29   | |
| 12  | 1,2,3,4-Tetrahydro naphthalene           | 9.85| 1164| 2.80| –       | |
| 13  | Azulene                                  | 10.25| 1298| 3.47| –       | |
| 14  | Dodecane                                 | 10.39| 1200| –   | 5.4    | |
| 15  | 2,6-Dimethyl undecane                    | 10.64| 1215| 0.16| –       | |
| 16  | *β*-Cyclocitral                          | 10.88| 1217| 1.91| 0.57   | |
| 17  | *p*-Anisaldehyde                         | 11.52| 1247| 1.52| –       | |
| 18  | Cinnamaldehyde                           | 11.81| 1267| 0.73| –       | |
| 19  | Nonanoic acid                            | 11.95| 1267| 0.73| 1.1    | |
| 20  | Anethol                                  | 12.05| 1282| 4.60| –       | |
| 21  | Thymol                                   | 12.16| 1289| –   | 2.65   | |
| 22  | 2-Methyl naphthalene                     | 12.21| 1281| 3.85| –       | |
| 23  | 1-Methyl naphthalene                     | 12.51| 1298| 2.89| –       | |
| 24  | 2,6,10-Trimethyldecane                   | 13.45| 1258| –   | 1.31   | |
| 25  | Eugenol                                  | 13.24| 1356| 7.17| –       | |
| 26  | Tetradecane                              | 13.84| 1400| –   | 3.87   | |
| 27  | 1,7-Dimethyl naphthalene                 | 14.08| 1426| 3.24| –       | |
| 28  | *α*-Ionone                               | 14.38| 1426| 2.36| –       | |
| 29  | *β*-Ionone                               | 15.36| 1485| 8.02| 6.65   | |
| 30  | Hexadecane                               | 16.88| 1600| 4.11| 1.55   | |
| 31  | Heptadecane                              | 18.31| 1700| –   | 2.25   | |
| 32  | 3-Methyl heptadecane                     | 18.91| 1737| –   | 0.65   | |
| 33  | 2-Methyl heptadecane                     | 19.17| 1765| –   | 0.86   | |
| 34  | Octadecane                               | 19.62| 1800| 2.04| 2.34   | |
| 35  | 2,6,11,15-Tetramethyl hexadecane          | 20.26| 1810| 0.34| –       | |
| 36  | Nonadecane                               | 20.93| 1900| 1.29| 0.76   | |
| 37  | Methyl palmitate                         | 21.80| 1927| 2.15| 0.71   | |
| 38  | Heneicosane                              | 23.31| 2100| 0.54| 0.75   | |
| 39  | Phytol                                   | 23.51| 2100| 0.36| –       | |
| 40  | Docosane                                 | 24.44| 2200| 0.45| 0.53   | |
| 41  | Benzy l butyl phthalate                  | 26.20| 2387| 1.18| –       | |
| 42  | Pentacosane                              | 27.53| 2500| 0.65| –       | |
| Sum |                                         |     |    | 78.83| 91.80  | |
| % of oxygenated compounds | 54.29% | 72.20% |
our study emphasised on the antimicrobial activity of the different oils against different Gram-
positive and Gram-negative bacteria as well as different dermatophytes.

2.3. Cytotoxic activity
The cytotoxic activity of the NCA and NEA oils was evaluated using viability assay on human
cancer cell lines colon (HCT-116), breast (MCF7) and lung (A-549) and the IC\textsubscript{50} values were
calculated (Table S3). NEA and NCA oils were active against lung carcinoma with IC\textsubscript{50} of 43.5
and 46.2 µg/mL, and breast carcinoma with IC\textsubscript{50} of 44 and 47.9 µg/mL, respectively. This may
be attributed to the presence of phytol, α-ionone and β-ionone which have been reported with
cytotoxic activities (Dasgupta & Humphrey 1998; Sua et al. 2013). NCA and NEA oils were
inactive against human colon cell line (HCT-116).

3. Conclusion
This study is the first report on the composition of volatile constituents, the antimicrobial and
antidermatophyte as well as cytotoxic activities of the aerial parts of NC and NE cultivated in
Egypt. NEA oil has displayed potent antibacterial and antifungal activities and could be a useful
alternative for the treatment of dermatophytosis. NEA oil also showed potent cytotoxicity
against three cell lines. We hope that our results will provide a starting point for investigations
designed for a new natural antimicrobial essential oil of this plant species. Additional in vivo
studies and clinical trials will also be needed to justify and further evaluate the potential of this
oil as an antimicrobial agent in topical or oral applications.

Supplementary material
Experimental details relating to this paper are available online at http://dx.doi.
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Disclosure statement
No potential conflict of interest was reported by the authors.

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