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Full Length Research Paper

Chemical analysis of *Magnolia liliflora* essential oil and its pharmacological function in nursing pregnant women suffering from decubitus ulcer

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The composition of the essential oil of *Magnolia liliflora* has been analyzed by GC-MS. The main components identified were: β-Pinene (21.16%), eucalyptol (16.59%), bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)- (9.86%), 3-Cyclohexene-1-methanol, à,à,4-trimethyl-, (S)- (7.13%), 3-cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)- (6.51%), α-pinene (6.31%), camphene (6.03%). Pharmacological function of *M. liliflora* essential oil was evaluated by examing its effect on serum IL-4, IFN-γ and decubitus ulcer area in pregnant women suffering from decubitus ulcer. Results showed that *M. liliflora* essential oil could reduced serum IL-4 level and decubitus ulcer area. This indicated that *M. liliflora* essential oil is effective for nursing pregnant women suffering from decubitus ulcer.

Key words: *M. liliflora* essential oil, pregnant women, nursing, ulcer.

INTRODUCTION

There are increasing number of investigations on plant extract activities of immune enhancement and anti-micro-organism. Essential oils, methanol and ethanol extracts are known to possess antimicrobial activity (Alzoreky and Nakahara, 2003; Abu-Shanab et al., 2004; Shahverdi et al., 2007; Behrooozi et al., 2010; Ma et al., 2010; Yin et al., 2010), essential oils has been traditionally used for respiratory tract infections, and are used these days as ethical medicines for colds (von Schindl, 1972; Federspil et al., 1997).

M. liliflora Desr (Magnoliaceae) is a 3 to 4 m deciduous shrub propagated and distributed in many parts of East Asia and North America. It has been reported that *M. liliflora* has the beneficial effects on several ailments such as analgesic, anodyne, carminative, febrifuge, sedative, and tonic (Duke and Ayensu, 1985). Previously, we reported the chemical composition and antibacterial properties of the essential oil and various organic extracts of *M. liliflora* against foodborne and spoilage bacteria (Bajpai et al., 2008).

The decubitus ulcer represents a defect that can extend from the epidermis through the underlying tissue, even reaching to bone. Decubitus ulcers constitute a health problem that affects any institution dedicated to health care. The prevalence of this problem has not been established precisely and ranges from 1 to 18% of hospitalized patients (Shannon and Skorga, 1989; Gosnell et al., 1992; Olson et al., 1996) and from 3 to 28% of those admitted to long-term care units (Weiler et al., 1990; Frantz et al., 1995; Bergstrom et al., 1996; Huang et al., 2010). It is estimated that more than 1 million people in hospitals and clinics in the United States may have pressure ulcers (National Pressure Ulcer Advisory Panel, 1989). IFN-γ and IL-4 are prototype Th1 and Th2 cytokines, respectively, whose antagonistic actions against each other have been widely reported (Mosman and Sad, 1996). They reciprocally regulate not only Th1 and Th2 cell survival and the subsequent differentiation, but also activation and differentiation of B cells as well as monocytes (Constant and Bottomly, 1997; Snapper and Paul, 1987; Cao et al., 1989). Specific target molecules that are counter-regulated by IFN-γ and

IL-4 play important roles in mediating Th1 or Th2 immune response. In humans and, particularly, in mice, resistance to infection is associated with a Th1-type immune response with production of the cytokines IL-4 and IFN-γ.

Decubitus ulcer may occur in pregnant women who are bedridden for prolonged periods of time. In this study, we extracted essential oil from dried flower of *M. liliflora* and analysed chemical composition of the essential oil. Then, we evaluated pharmacological function in nursing pregnant women suffering from decubitus ulcer.

MATERIAL AND METHOD

Plant material

The dried flower of *M. liliflora* was collected during September 2009 from Taizhou city, China and the identity was confirmed by Professor Wang in the School of Pharmacy, Taizhou University. A voucher specimen (20090913) has been retained in nursing research laboratory in TaiZhou University.

Extraction and identification

The air-dried material (500 g) was hydrodistilled in a Clevenger-like apparatus for 2 h and the essential oil collected and analyzed by GC–MS.

GC/MS analysis

GC/MS analysis was performed on a QP-5000 system (Shimadzu, Japan) by electron ionization (EI) at 70 eV. GC separation was performed on a capillary column (0.25 mm i.d × 30 m, film 0.5 mm) of HP-5MS (Hewlett Packard, USA). Each extract (1 ml) was injected using the splitless injection method. The column temperature was initially 50°C, then raised to 200°C at a rate of 10°C/min, and finally held at 200°C for 5 min. The injection port was set at 120°C. Helium gas was used as the carrier at a flow rate of 0.6 ml/min (Nitta et al., 2006).

Study population and design

A total of 37 pregnant women were recruited in affiliated hospital of TaiZhou University in October in 2009. These women were suffering from decubitus ulcer. Decubitus ulcer of all women were handled twice daily for 6 weeks. Area of decubitus ulcer and levels of blood IL-4 and IFN-γ were measured at an interval of 1 week.

Sera were separated and aliquots were stored at -20ºC for later measurement of cytokines. Human IL-4 and IFN-γ were measured by a cytokine specific sandwich avidin-biotin ELISA using mouse monoclonal antibody (mAb) pair (native capture mAb and biotinylated detecting mAb, bender-med-system Vienna, Austria). In brief, flat-bottom polystyrene micro-plates was precoated with anti human cytokines were employed. Before cytokine assay, micro-plates had been washed and blocked with PBS. Serial dilutions of cytokine standards IL-4 and IFN-γ were performed with the assay buffer (PBS with 1% between 20 and 10% BSA) were produced and add to single well. Bound cytokines were detected using biotinylated antibody followed by poly-streptavidin -HRP buffer and developed with 1-step TMB coloration. Color development was measured at 450 nm.

Statistical analyses

Results were expressed as means ± SEM for 37 people. Statistical evaluation of the data was carried out by the parametric Student t test. The calculations were performed using STATISTICA 6.0 (for Windows, StatSoft Inc software, Tulsa, OK). The limit of statistical significance was set at $P < 0.05$ between the different groups.

RESULT AND DISCUSSION

Chemical composition of *M. liliflora* essential oils

The yield of essential oils obtained by steam distillation from *M. liliflora* was 5.98%. 61 component chromatographic peaks of the oil were detected and 57 components of the oil were identified. The content of the identified components was more than 94.03% of the oil. The identified components where the content is more than 0.01% are given in Table 1. The contents of each kind of component are listed in Table 1.

β-Pinene (21.16%) and eucalyptol (16.59%) were the most abundant components and comprised 40% of the oil. The major compounds obtained in this oil still included Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)- (9.86%), 3-Cyclohexene-1-methanol, à,à,4-trimethyl-, (S)- (7.13%), 3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)- (6.51%), α-Pinene (6.31%), Camphene (6.03%), terpineol, cis-á- (3.28%), Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, (1S-endo)- (2.58%), Borneol (2.18%), 2-Naphthalenemethanol, decahydro-α,α,4a-trimethyl-8-methylene-, [2R-(2α,4aα,8aβ)]- (1.83%), Caryophyllene (1.45%).

Pharmacological function of *M. liliflora* essential oil in nursing pregnant women suffering from decubitus ulcer

IL-4 is a pleiotropic and multifunctional cytokine produced by activated T cells, mast cells and basophils (Nelms et al, 1999). In addition, eosinophils and a specialized subset of T cells (NK1.1+) have also been reported to produce IL-4 (Chen and Paul, 1997; Dubucquoi et al., 1994). IL-4 plays a critical role in regulating the outcome of an immune response by facilitating type 2 Th cell differentiation and suppressing the differentiation of IFN-γ-producing CD^{4+} T cells, thereby favoring humoral immune responses (Hsieh et al., 1992; Seder et al., 1992). The other important function of IL-4 is the regulation of immunoglobulin class-switching. It induces class-switching to IgE and IgG4 (in human B cells) and to IgE and IgG1 (in mouse B cells) (Gascan et al., 1991; Coffman et al., 1986; Vitetta et al., 1985). The increased levels of IgE antibodies that are reactive to the allergens associated with atopic diseases suggest a pre-eminent role in the regulation of such conditions for IL-4. In addition to its direct involvement in regulating immune responses, IL-4 also exerts a wide variety of effects on hematopoietic and nonhematopoietic cells.

Table 1. Chemical composition of *M. liliflora* essential oil.

S/No.	Compound	RT (min)	Percentage (%)
1	α-Pinene	4.60	6.31
2	Camphene	4.81	6.03
3	β-Pinene	5.15	21.16
4	Eucalyptol	5.96	16.59
5	Terpineol, cis-á-	6.50	0.99
6	Terpineol, cis-á-	6.96	3.28
7	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	7.86	9.86
8	Borneol	8.20	2.18
9	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	8.45	6.51
10	3-Cyclohexene-1-methanol, à,à,4-trimethyl-, (S)-	8.70	7.13
11	Bicyclo[3.1.1]hept-2-ene-2-methanol, 6,6-dimethyl-	8.78	0.52
12	1,7-Octadien-3-ol, 2,6-dimethyl-	9.26	0.34
13	2,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-	9.76	0.24
14	trans-2-Caren-4-ol	10.20	0.25
15	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, acetate, (1S-endo)-	10.52	2.58
16	3-Cyclohexene-1-methanol, à,à,4-trimethyl-, acetate	11.81	0.21
17	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1à,2á,4á)]-	12.81	0.33
18	Benzene, 1,2-dimethoxy-4-(2-propenyl)-	12.99	0.08
19	Caryophyllene	13.51	1.45
20	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (Z)-	14.14	0.12
21	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	14.25	0.38
22	Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1à,4aà,8aà)-	14.70	0.14
23	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-	14.80	0.23
24	1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3aà,3bá,4á,7à,7aS*)]-	14.86	0.40
25	Eudesma-4(14),11-diene	14.98	0.19
26	Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1à,3aá,4à,7á)]-	15.16	0.22
27	Naphthalene,1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1à,4aà,8aà)-	15.23	0.13
28	Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-, (S)-	15.36	0.09
29	Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-,(1α,4aα,8aα)-	15.56	0.21
30	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	15.75	0.52
31	α-Calacorene	16.21	0.06
32	Cyclohexanemethanol, 4-ethenyl-à,à,4-trimethyl-3-(1-methylethenyl)-, [1R-(1à,3à,4á)]-	16.35	0.60
33	Caryophyllene oxide	16.46	0.11
34	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-	16.58	0.06
35	9-Methoxycalamenene	16.67	0.04
36	(-)-Spathulenol	17.04	0.37
37	Caryophyllene oxide	17.18	0.87
38	10-Heptadecen-8-ynoic acid, methyl ester, (E)-	17.29	0.05
39	3,4,4-Trimethyl-3-(3-oxo-but-1-enyl)-bicyclo[4.1.0]heptan-2-one	17.39	0.10
40	Cedrol	17.58	0.15
41	Calarene epoxide	17.73	0.36
42	Aromadendrene oxide-(2)	17.93	0.08
43	Cubenol	18.10	0.10
44	β-Guaiene	18.19	0.21

Table 1. Contd.

45	.tau.-Cadinol	18.39	0.39
46	ç-Himachalene	18.52	0.13
47	2-Naphthalenemethanol, decahydro-α,α,4a-trimethyl-8-methylene-, [2R-(2α,4aα,8aβ)]-	18.67	1.83
48	2-Naphthalenemethanol, 1,2,3,4,4a,5,6,8a-octahydro-α,α,4a,8-tetramethyl-, (2α,4aα,8aα)-	18.72	1.12
49	5-Azulenemethanol, 1,2,3,3a,4,5,6,7-octahydro-à,à,3,8-tetramethyl-, [3S-(3à,3aá,5à)]-	18.95	0.08
50	Isoaromadendrene epoxide	19.05	0.06
51	Tricyclo[5.2.2.0(1,6)]undecan-3-ol, 2-methylene-6,8,8-trimethyl-	19.37	0.25
52	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (E,E)-	20.04	0.85
53	2,6,10-Dodecatrienal, 3,7,11-trimethyl-	20.47	0.10
54	2,5,5,8a-Tetramethyl-4-methylene-6,7,8,8a-tetrahydro-4H,5H-chromen-4a-yl hydroperoxide	23.10	0.02
55	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	24.72	0.04
56	Hexadecanoic acid, trimethylsilyl ester	26.37	0.14
57	Tetradecane, 2,6,10-trimethyl-	26.53	0.02

Figure 1 showed that serum IL-4 level in pregnant women studied decreased with prolonged time after *M. liliflora* essential oil treatment. The results were found statistically significant ($P < 0.05$). At the 6th week, serum IL-4 level was reduced (32%).

Interferon (IFN)-γ is a pleiotropic cytokine endowed with potent immunomodulatory effects on a variety of immune cells *in vitro* and *in vivo* (Young and Hardy, 1995; Billiau, 1996). This cytokine exerts its multiple biological activities by controlling either positively or negatively the expression of many genes and proteins. Although IFN-γ was originally defined as an antiviral factor produced by stimulated lymphocytes (Wheelock, 1965; Ajmal and Ahmed, 2009), it subsequently became clear that this cytokine can exhibit broader effects on several branches of the immune system (Young and Hardy, 1995; Billiau, 1996). In particular, IFN-γ exerts important activities on both monocyte\macrophages and lymphocytes, which generally result in macrophages activation and T cell differentiation towards a Th-1 type of immune response (Nathan et al., 1984; Gajewski and Fitch, 1988). The importance of IFN-γ to the function of several cell types of the murine immune system has been clearly demonstrated by the generation of mice with a targeted disruption of IFN-γ (Dalton et al., 1993) or IFN-γ receptor (Huang et al., 1993) genes.

Figure 2 showed that serum IFN-γ level in pregnant women studied decreased with prolonged time after *M. liliflora* essential oil treatment. The results were found statistically significant ($P < 0.05$). At the 6th week, serum IFN-γ level was reduced (32%).

In brief, the hydrodistillated oil of *M. liliflora* is consisted of oxygenated mono- and sesqueterpenes, and mono- and sesqueterpene hydrocarbons (Bajpai et al., 2008). In recent years, several researchers have reported the mono- and sesquiterpenes, and mono- and sesquiterpene hydrocarbons as the major components of essential oils, which have enormous potential to strongly inhibit microbial pathogens (Gudzic et al., 2002; Cakir et al., 2004; Sun et al., 2009). A pressure ulcer is a localized

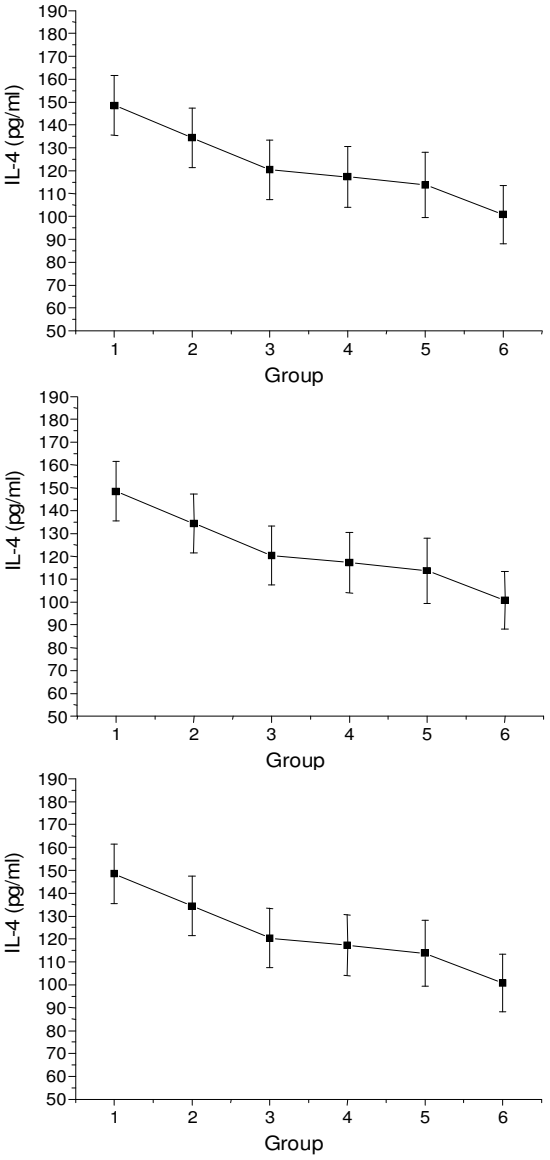

Figure 1. Effect of *M. liliflora* essential oil on serum IL-4 level.

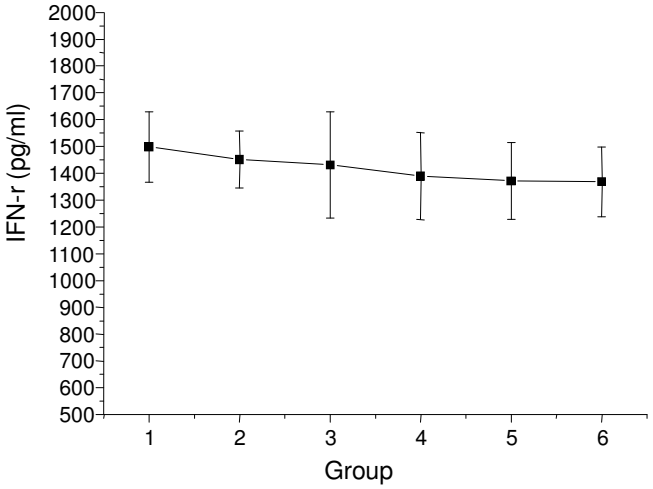

Figure 2. Effect of *M. liliflora* essential oil on serum IFN-γ level.

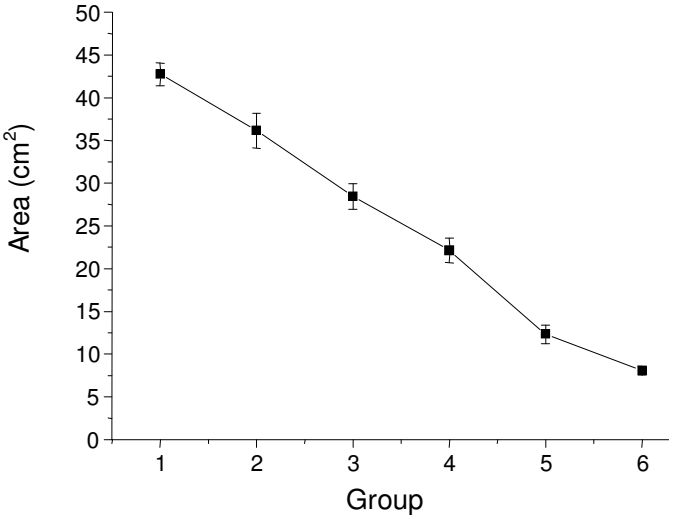

Figure 3. Effect of *M. liliflora* essential oil on the area of decubitus ulcer.

area of tissue damage caused by excess pressures, shearing, or friction forces that occur in people who cannot reposition themselves to relieve pressure on boney prominences (Moore and Cowman, 2007). A pressure ulcer may also be known as a bedsore, pressure sore, or decubitus ulcer. Anyone can get a pressure ulcer, but some people are more likely to develop one than others. People with a pressure ulcer are also at risk of developing another pressure ulcer. According to the author's knowledge, effect of *M. liliflora* essential oil on decubitus ulcer of pregnant women was first reported.

In contrast to control (0 week), there was a significant decrease (14 to 81%) in the area of decubitus ulcer when the *M. liliflora* essential oil was administered to the decubitus ulcer in pregnant women studied. This indicated that *M. liliflora* essential oil is effective in curing decubitus ulcer (Figure 3).

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