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Antifungal Properties and Chemical Analysis of Essential Oil from *Vitex negundo* Seeds

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Authors' contributions

This work was carried out in collaboration between all authors. Author CJZ designed the study, wrote the protocol. Authors CJZ and HWA performed the study and wrote the manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: To investigate the constituents and antimicrobial activity of essential oil from *Vitex negundo* seeds.
Study Design: The essential oil of *Vitex negundo* seeds was prepared by hydrodistillation. GC/MS technique was used to determine the volatile constituents in the oil. A total of 13 isolates of bacteria and fungi were employed to evaluate the antimicrobial activity of the oil.
Place and Duration of Study: Department of Pharmacognosy, School of Pharmacy, Second Military Medical University, between April 2010 and September 2010.
Methodology: Essential oil from *Vitex negundo* seeds were obtained by hydrodistillation, with constituents analyzed by GC-MS. Antimicrobial activity of the oil was evaluated against both Gram-positive and Gram-negative bacteria and fungi.
Results: A total of forty-two components, representing 91.36% of the oil, were identified. *n*-Hexadecanoic acid (17.68%), eudesm-4(14)-en-11-ol (12.39%) and caryophyllene oxide (10.79%) were found to be the major constituents. The essential oil exhibited significant antifungal activity against *Candida albicans* at MIC 4.0 µg/ml.
Conclusion: The essential oil of *Vitex negundo* seeds showed potential antifungal activity and can be of potential use in pharmaceutical fields.

Keywords: Vitex negundo; seeds, essential oil; GC/MS analysis; antifungal.

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1. INTRODUCTION

Repeated use of antibiotic and insufficient control of the infectious diseases have been aggravating the problem of multidrug resistance since recent decades, with human health exposed to the sharply increasing threat of drug resistant strains [1]. Therefore, substantial researches for anti-infective drugs derived from plants resources of new prototype are now highly focused on and desperately needed to solve the global problem of resistance to antimicrobial agents.

Essential oils are mixtures of different volatile aromatic compounds extracted from plants. Since the discovery of their antifungal and antimicrobial properties, preparations of plant essential oils have been applied in pharmacology, medical microbiology, phytopathology, and food preservation [2,3]. With the increasing public concern, studies in search of promising essential oils with high effectiveness and low toxicity against a variety of microorganisms are getting more and more attention.

Vitex negundo (VN) (Verbenaceae) is an aromatic plant that flourishes abundantly in wastelands and widely distributes in tropical to temperate regions, native of South Asia, China, Indonesia, and the Philippines, up to an altitude of 1500m. All parts of VN have been commonly used as folk medicine. The extract of VN leaves possessed anti-inflammatory, analgesic, hypouricaemic, anti-hyperglycemic and antibacterial activity [4-7]. Anti-oxidant [8] and antiandrogenic properties [9,10] were reported from the flavonoid-rich fraction of the seeds. VN also found use for CNS depressant [11], anti-histamine release [12], hepato-preventive [13] and antineoplastic purposes [14]. Flavonoids [9,15], iridoid glycosides [14], lignans [8], alkaloids [16], and terpenoids [17] are the major constituents isolated from this plant according to previous phytochemical studies.

There have been few studies conducted on the chemical compositions and pharmacological effects of the essential oil from *V. negundo* seeds [18,19]. Previous studies revealed the major presence of 1,8-cineole, p-menth-1-en-8-ol, ethyl palmitate [18], β-selinene, α-cedrene, and germacrene D [19] in the essential oil of *V. negundo* seeds, which exhibited antibacterial [19] and immunomodulatory effect [20] in vitro.

Although the chemical composition of the essential oil of *V. negundo* seeds was previously studied, little research has so far been conducted to elucidate the antimicrobial activity of the seeds essential oil. Thus in the present work, we report herein the compositions and antibacterial and antifungal effects of the essential oil from *V. negundo* seeds collected in China.

2. MATERIALS AND METHODS

2.1 Plant Material

The seeds of *Vitex negundo* L. (Chinese name“Huang-Jing-zi”), which was identified by Prof. Lu-ping Qin (School of Pharmacy, Second Military Medical University), was obtained from the Wanglang National Nature Reserve, Sichuan province. Voucher specimens (#2006-168) have been deposited in the herbarium of the Department of Pharmacognosy.

2.2 Extraction of Essential Oil

A total of 100 g sample was powdered and extracted by hydro distillation method [20] for 5 hours to yield 0.1% (v/w) clear yellowish oil with a bitter odor, which was dried with anhydrous sodium sulfate and stored at 5°C for further experiments.

2.3 GC-MS Analysis

Chromatography was performed on a Thermo Focus DSQ gas chromatograph with a mass-selective detector with electron impact ionization. The prepared oil was separated using a VF-5MS capillary column of 30 m×0.25mm with a phase thickness of 0.25µm from Superlco, which was inserted directly into the ion source of the MS system. The temperature program used for analysis was as follows: the initial temperature was 60°C which was maintained for 2 min; to 300°C at 10°C /min and then maintained for 10min. Helium (99.999%) was the carrier gas at a flow-rate of 1.0 ml/min. The split rate was 20:1 and inlet volume was 1.0 µl.

The electron impact ionization conditions were: ion energy 70eV and the mass range scanned was 29-450 a.m.u in the full-scan acquisition mode. Compounds were identified using the NIST Mass Spectral Search Program (National Institute of Standards and Technology, Washington, DC,USA).

2.4 Anti-*Pyrucularia oryzae* Activity

Previous studies revealed considerable correlation of anti-*Pyrucularia oryzae* activity with antifungal activity of many Chinese traditional and herbal drugs [21]. In our work, *Pyrucularia oryzae* P-2b was used as an indicator for preliminary antimicrobial bioactivity screening of the essential oil from *Vitex negundo* seeds, of which the MMDC (minimum morphological deformation concentration) was determined as 125µg/ml (Table 2).

2.5 Determination of Minimum Inhibitory Concentration (MIC) Against Microbial Stains

The seed oil was tested against 13 isolates of microorganisms, including two reference strains: *Candida albicans* ATCC-76615 and *Cryptococcus neoformans* ATCC-32609, and eleven clinically isolated strains: *Candida parapsilosis*, *Candida tropicalis*, *Trichophyton rubrum*, *Fonsecaea compacta*, *Microsporum gypseum*, *Aspergillus fumigatus*, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus faecalis, Micrococcus catarrhalis,* and *Pseudomonas aeruginosa*.

A broth microdilution testing procedure, proposed by the National Committee for Clinical Laboratory Standards (NCCLS) [21,22], was used to determine the minimum inhibitory concentration (MIC). A serial doubling dilution of the oil supplemented with Tween 80 at final concentration of 0.5% (V/V) was prepared in a 96-well tissue culture plates ranging from 0.125 to 64 µg/ml.

The final concentration of each stain was adjusted to $1\sim5\times10^3$ CFU/mL using RPMI 1640 media buffered with MOPS (3-[N-Morpholino] propanesulfonic acid) (Sigma Chemical Co.). Bacteria were incubated at 37°C for 24h, while fungi were co-incubated with the oil at 35°C for 96h. The MIC was defined as the lowest concentration of the essential oil at which 80%

of the tested strain was inhibited, recorded spectrophotometrically by the decrease of OD value.

3. RESULTS AND DISCUSSION

A total of 42 components identified comprised 91.36% of the essential oil from *Vitex negundo* seeds. Quantitative and qualitative analytical results showed that the oil consisted mainly of oxygenated sesquiterpenes and fatty acids. *n*-Hexadecanoic acid (17.68%), eudesm-4(14)-en-11-ol (12.39%) and caryophyllene oxide (10.79%) was found to be the major constituents as summarized in Table 1. Both of the compositions and their percentage of the oil varied considerably with previous studies reporting high content of 1, 8-cineole, p-Menth-1-en-8-ol, ethyl palmitate [18], β-selinene, α-cedrene, and germacrene D [19], which might be the result of different origins of herbs, different collected seasons, different living environment conditions, or different chromatographic conditions in GC-MS analysis.

Subsequently, we detected the MMDC value of the seed oil (Table 2) against *Pyrucularia oryzae*, an indicator for the preliminary screening of active compounds or extracts with antifungal properties. Our data indicated the antifungal potential of the oil and further studies were conducted to evaluate the antimicrobial activity of the seed oil against 13 microorganisms using broth microdilution method to determine its MICs. Our results (Table 3) showed that the essential oil exhibited higher potency and lower MICs against the tested fungi than those against bacteria, which was consistent with the indication of its anti-*Pyrucularia oryzae* activity. *Candida albicans* was the most sensitive to the essential oil of *Vitex negundo* seeds with the lowest MIC at 4.0μg/ml. It was thought that antifungal activity of the essential oil could be mostly due to the abundance of sesquiterpenes, diterpenes and their derivates in the present work, including caryophyllene oxide [23], cubenol [24], τ-eudesmol [25], (13R)-labd-14-ene-8,13-diol [26] and possibly some minor components that might be involved in some type of synergism with the active compounds. However, lack of monoterpenes, such as *β*-pinene and 1, 8-cineole with pronounced antimicrobial potentials [27,28], the oil showed weak bacteriostatic activity in our experiment. In a previous study, the fruits oil of *Vitex negundo* showed β-selinene, α-cedrene and germacrene D as the main constituents exhibiting promising antibacterial activity against *B. subtilis* and *E. coli* and *S. aureus* [19], which agreed with the former findings that the essential oil with higher content of monoterpenes exhibited better antibacterial activities [29]. Although the seeds oil investigated in our study possessed weak antibacterial activity, it showed potential antifungal activity, especially against *Candida albicans*, with promising clinical utilization in fungal infection prevention.

Table 1. Analytical results of volatile constitutions from *Vitex negundo* seeds

Compounds	RC* (%)	RT*(min)	Formula
Hexanal	1.17	3.10	$C_6H_{12}O$
1-iodo-Pentane	0.10	4.63	$C_5H_{11}I$
p-Menth-1-en-8-ol	0.17	7.76	$C_{10}H_{18}O$
2-Isopropyl-cyclohexanone	0.32	8.19	$C_9H_{16}O$
1-Tridecanol	0.17	8.42	$C_{13}H_{28}O$
(*E*,*E*)-2,4-Decadienal	0.84	8.92	$C_{10}H_{16}O$
2-Ethyl-2-hexen-1-ol	0.63	9.20	$C_8H_{16}O$
Geraniol acetate	0.16	9.40	$C_{12}H_{20}O_2$
(*E*)-3-Decen-2-ol	0.18	9.48	$C_{10}H_{20}O$
9-Oxononanoic acid	0.91	10.31	$C_9H_{16}O_3$
Eudesma-4(14),11-diene	2.15	10.57	$C_{15}H_{24}$
Cadina-1,3,5-triene	0.59	10.80	$C_{15}H_{22}$
(1S,2S,4R)- (-)-α,α-Dimethyl-1-vinyl-*o*-menth-8-ene-4-methanol	0.94	11.00	$C_{15}H_{26}O$
-(-)-Spathulenol	2.43	11.30	$C_{15}H_{24}O$
Caryophyllene oxide	10.79	11.37	$C_{15}H_{24}O$
Viridiflorol	0.49	11.54	$C_{15}H_{26}O$
1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	0.47	11.59	$C_{15}H_{24}O$
Cubenol	1.31	11.68	$C_{15}H_{26}O$
τ-Eudesmol	0.35	11.72	$C_{15}H_{26}O$
(+)-Ledene	0.95	11.76	$C_{15}H_{24}$
τ-Himachalene	0.94	11.89	$C_{15}H_{24}$
Eudesm-4(14)-en-11-ol	12.39	11.94	$C_{15}H_{26}O$
Isoaromadendrene epoxide	1.81	12.38	$C_{15}H_{24}O$
formic acid 3,7,11-Trimethyl-1,6,10-dodecatrien-3-yl ester	0.15	12.45	$C_{16}H_{26}O_2$
18-Norabietane	0.63	12.62	$C_{19}H_{34}$
Vitamin A1 (Retinol)	2.23	12.92	$C_{20}H_{30}O$
Hexahydrofarnesyl acetone	0.83	13.08	$C_{18}H_{36}O$
Farnesyl bromide	0.32	13.19	$C_{15}H_{25}Br$
Arachidonic acid methyl ester	0.85	13.79	$C_{21}H_{34}O_2$
n-Hexadecanoic acid	17.68	13.91	$C_{16}H_{32}O_2$
(13R)-Labd-14-ene-8,13-diol	5.65	14.17	$C_{20}H_{36}O_2$
5-(1-Isopropenyl-4,5-dimethylbicyclo[4.3.0]nonan-5-yl)-3-methyl-2-pentenol acetate	6.26	14.22	$C_{22}H_{36}O_2$
(13R)-Labd-14-ene,8,13-epoxy,	0.38	14.38	$C_{20}H_{34}O$
13*β*-Methyl-13-vinyl-podocarp-8 (14)-ene	0.36	14.43	$C_{20}H_{32}$
7-Isopropyl-1,1,4a-trimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene	2.17	14.73	$C_{20}H_{30}$
Labd-7-en-15-oic acid methyl ester	0.56	14.92	$C_{21}H_{36}O_2$
(*Z*,*Z*)-9,12-Octadecadienoic acid	5.88	15.02	$C_{18}H_{32}O_2$
(E)-9-Octadecenoic acid	5.51	15.05	$C_{18}H_{34}O_2$
Octadecanoic acid	0.94	15.18	$C_{18}H_{36}O_2$
14-Isopropylpodocarpa-8,11,13-triene-7,13-diol	0.32	16.22	$C_{20}H_{30}O_2$
13-Isopropyl-podocarpa-8,11,13-trien-16-ol,	0.21	16.27	$C_{20}H_{30}O$
7-Oxodehydroabietic acid methyl ester	0.17	16.78	$C_{21}H_{28}O_3$

**RC: relative content; RT: retention time*

Table 2. Anti-*Pyrucularia oryzae* activity of essential oil from *Vitex negundo* seeds

Concentration(μg/ml)	1000	500	250	125*	62.5	31.3	15.6	7.8
Activity agaist *Pyrucularia oryzae*	×	×	×	+++	-	-	-	-

**MMDC; × means inhibition; +++ means morphological deformation; - means no activity*

Table 3. Antimicrobial activity of essential oil from *Vitex negundo* seeds

Treatment	Minimum inhibitory concentration (MIC) in μg/ml against												
	Bacteria*					Fungi*							
	1	2	3	4	5	6	7	8	9	10	11	12	13
Essential oil	>64	64	>64	>64	>64	4.0	64	>64	64	32	>64	64	>64
Fluconazole	ND	ND	ND	ND	ND	1.0	0.5	2.0	2.0	1.0	2.0	1.0	2.0
Ampicillin	0.09	0.02	50.0	0.02	0.02	ND	ND	ND	ND	ND	ND	ND	ND

**1,Staphylococcus aureus; 2, Escherichia coli; 3, Pseudomonas aeruginosa; 4, Streptococcus faecalis; 5, Micrococcus catarrhalis; 6, Candida albicans (ATCC-76615); 7, Cryptococcus neoformans (ATCC-32609); 8, Candida parapsilosis; 9, Candida tropicalis; 10, Trichophyton rubrum; 11, Fonsecaea compacta; 12, Microsporum gypseum; 13, Aspergillus fumigatus; ND, not done*

4. CONCLUSION

Our study revealed the potential antifungal activity of the essential oil from *Vitex negundo* seeds for the first time, especially against several tested fungi strains, such as *Candida albicans* and *Trichophyton rubrum*, demonstrating promising use in the development of antifungal agents.

In addition, even though the essential oil of *Vitex negundo* seeds is surrounded with antifungal claims, the underlying mechanisms involved in its pharmacological effects are lacking. More scientific data are required before its increase clinical utilization can be given full confidence and further researches should be taken afterwards.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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