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CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *Sagittaria trifolia*

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The essential oil of Sagittaria trifolia, *a well-known famous medicinal foodstuff in China, was analyzed for the first time using GC-MS. Twenty-eight constituents were identified. The major components of the oil were hexahydrofarnesyl acetone (62.3%), tetramethylhexadecenone (5.8%), myristaldehyde (4.7%),* n-*pentadecane (2.9%), and 2-hexyldecanol (2.9%).The antimicrobial activity of the essential oil was evaluated against seven microorganisms, including two clinically isolated strains and five reference strains, using microbiological cylinder plate assay and broth microdilution methods. The results showed that the oil had a significant antimicrobial effect on four of them. This antimicrobial activity can partly explain why the oil is used medicinally during childbirth and for skin diseases in Chinese traditional medicine.*

Key words: *Sagittaria trifolia,* essential oil, GC/MS analysis, antimicrobial activity.

The genus *Sagittaria* L., the second largest genus in the family Alismataceae, comprises about 30 species with a worldwide distribution, and there are 8 species in China. Among those species, *S. trifolia* occurs in almost all Asian countries [1].

Currently, the chemical constituents and pharmacological evaluations of some *Sagittaria* species have been reported. For example, from the fresh tuber of *S. trifolia*, eleven diterpenes, two diterpene glucosides, and a nitroethylphenol glycoside were isolated [2]. Among the diterpene constituents, four ketones, (trifoliones A, B, C, and D) exhibited inhibitory effects on histamine release from rat mast cells [3]. From the fresh petioles of *S. montevidensis ssp montevidensis*, abietenes 7,13-abietadien-3-one and 8,11,13 -abietatrien-3-ol were isolated [4].

S. trifolia is not only a well-known herb but also a nutrient foodstuff. In traditional medicine, it has significant anti-skin infection effects and, unlike common clinic dermatosis medicine, as a food it is innocuous in appearance and use. However, the chemical composition and antimicrobial activity of aerial parts of *S. trifolia* have not been reported. As part of our research on the medicinal foodstuffs of China, we decided to investigate the volatile oil of the aerial parts of *S. trifolia*.

In the present paper, for the first time, we identify the chemical composition of the essential oil from the aerial parts of *S. trifolia* by the GC-MS method. Aerial parts of *S. trifolia* yielded 0.1% (v/w) of a yellowish oil with an aromatic odor. Thirty-nine compounds were isolated, of which 28 compounds were identified. The list of compounds identified in the oil sample is presented in Table 1 with their percentage content. The major components of the oil were long-chain hydrocarbons and ketones: hexahydrofarnesyl acetone (62.3%), tetramethylhexdecenone (5.8%), myristaldehyde (4.7%), *n*-pentadecane (2.9%), and 2-hexyldecanol (2.9%). Among the minor components, caryophyllene and its isomers (3.54%) were identified, which were previously been shown to have antimicrobial activity [5].

We also evaluate their antimicrobial properties using microbiological cylinder plate and broth microdilution methods. The oils showed significant antimicrobial effect on four strains (*M. flavus* ATCC 14698, *B. cereus* CMCCB 63301, *S. lutea*, and *S. aureus*). The data of DD (diameter of zone of inhibition (mm) including disc diameter of 6 mm), MIC (minimum inhibitory concentration), and MBC (minimum bactericidal concentration) are presented in Table 2.

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TABLE 1. Composition of the Essential Oil of *Sagittaria trifolia*

RI	Compound	%	RI	Compound	%
1000	*n*-Pentylfuran	0.3	1633	Tridecyl aldehyde	0.6
1220	*n*-Decaldehyde	0.4	1662	Hexahydrofarnesol	0.7
1415	*n*-Tetradecane	0.4	1695	2-Hexyldecanol	2.9
1437	Isocaryophyllene	0.9	1719	*n*-Heptadecane	1.3
1439	Longifolene	1.2	1737	Myristaldehyde	4.7
1451	Caryophyllene	0.8	1752	Tetramethylpentadecene	0.6
1472	*trans*-Geranylacetone	0.7	1788	Myristic acid	2.1
1478	Methylpentadecane	2.1	1821	*n*-Octadecane	0.6
1485	*β*-Caryophyllene	0.6	1862	Tetramethylpentadecenol	0.8
1514	*n*-Pentadecane	2.9	1871	Hexahydrofarnesyl acetone	62.3
1588	Dimethylundecenol	1.4	1915	2-Hexyl-1-decanol	1.2
1602	Pentadecene	1.0	1922	*n*-Nonadecane	0.9
1618	*n*-Cetane	1.1	1949	Tetramethylhexadecenone	5.8
1621	Caryophyllene oxide	0.9	1963	Phenanthrenol	0.8

RI: Retention indices on HP-5 capillary column.
%: Percentage of the content of each constituent in total essential oil.

TABLE 2. Antimicrobial Activity of the Essential Oil of *Sagittaria trifolia*

Microorganisms	DD[a]	MIC[b]	MBC[b]
M. flavus ATCC 14698	13.6	3.1	6.25
B. cereus CMCCB 63301	11.2	3.1	12.5
S. lutea	10.3	50	50
S. aureus	10.1	50	50
E. coli ATCC 11775	NA	NA	NA
P. aerugino ATCC 10145	NA	NA	NA
P. vulgaris ATCC 13315	NA	NA	NA

DD, diameter of zone of inhibition (mm) including disc diameter of 6 mm. NA, not active.
[a]Tested at a concentration of 6.00 mg/disc.
[b]Values given as mg/mL.

These results can partly explain why the leaf of “cigu” has traditional medicinal properties of anti-skin infection, since the essential oil from the aerial parts of *S. trifolia* has antimicrobial activity. The essential oil contains caryophyllene and its isomers (3.54%). Beta-caryophyllene, as a major compound of the essential oils of *Artemisia scoparia* Waldst, has been reported as demonstrating various degrees of bacteria growth inhibition [5]. They may also be active components and can contribute to the antimicrobial activity of the oil of *S.trifolia* as well.

EXPERIMENTAL

Samples of *S. trifolia* were collected from nanhui district in shanghai in China in May 2003. The samples were identified and the voucher was deposited in the Institute of Biodiversity Science, Fudan university (IBSFU), School of Life Sciences, Fudan University, China.

The volatile oil of the aerial parts of *S. trifolia* was obtained by hydrodistillation using a Clevenger-type apparatus for 3 h. The oil was subsequently dried over anhydrous sodium sulfate.

GC/MS analysis was performed on a combined GC/MS instrument (Finnigan Voyager, San Jose, CA, USA) using a HP-5 fused silica capillary column (30 m length, 0.25 mm diameter, 0.25 film thickness). A 1 μL aliquot of oil was injected into the column using a 10:1 split injection, with a set temperature at 250°C. The GC program was initiated by a column temperature set at 60°C for 2 min, increased to 250°C at a rate of 10°C/min, and held for 10 min. Helium was used as the carrier gas (1.0 mL/min). The mass spectrometer was operated in the 70 eV EI mode with scanning from 41 to 450 amu in 0.5 s, and the mass source was set at 200°C.

The compounds were identified by matching their mass spectral fragmentation patterns with those stored in the spectrometer database, using the National Institute of Standards and Technology Mass Spectral database (NIST-MS, 1998).

The essential oil was tested against seven microorganisms, of which the reference strains were *Micrococcus flavus* ATCC14698, *Bacillus cereus* CMCCB 63301, *Escherichia coli ATCC 11775, Pseudomonas aeruginosa ATCC 10145*, and *Proteus vulgaris ATCC 13315*, and the clinically isolated strains were *Sarcina lutea*, and *Staphylococcus aureus.*

The agar disc diffusion method was employed to determine the antimicrobial activity of the essential oils [6]. Briefly, a suspension of the tested microorganism (2×10^8 CFU/mL) was spread on solid media plates. Oxford cups (6 mm inner diameter and 7.8 mm outer diameter) were placed on the incubated plates, with each cup adding 300 μL of the diluted oil aliquots (20 mg/mL). The plates were held at 4°C for 2 h, followed by incubation at 37°C for 24 h. Then, the diameters of the inhibition zones were measured and expressed in millimeters. Each test was performed in three replicates and repeated twice. Modal values were selected.

A broth microdilution method was used to determine the MIC and MBC [7, 8]. The tests were performed in Mueller Hinton broth supplemented with Tween 80 (0.5% v/v), and the final concentration of each strain was adjusted to 5×10^4 CFU/mL. The culture medium, strains, and serial doubling dilutions of the oils ranged from 0.02 to 50 mg/mL and were prepared in 96-well microtiter plates, and the plates were incubated at 37°C for 24 h.

The MIC was defined as the lowest concentration of the essential oil at which the microorganism did not demonstrate visible growth. The MBC was defined as the lowest concentration of the essential oil at which the incubated microorganism was completely killed, with the microorganism growth indicated by the turbidity. Each test was performed in three replicates and repeated twice. Modal values were selected. The culture medium without added strains and oils served as the positive control in parallel experiments.

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