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ESSENTIAL OIL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF *Dichondra repens*

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Dichondra repens Forst is a Convolvulaceae plant. There are 5~8 species in the world and only one species in China. It is a traditional Chinese medicine, which has been known for a long time for its properties of heat-clearing, detoxicating, and damp elimination. It has been used in the treatment of diseases such as icterohepatitis, dysentery, and hydrops [1] in folk and clinical medicine. Extensive growth and cultivation of *Dichondra repens* Forst is now in progress and it has been exploited as a beverage food in China. Research [2] on the antibacterial activity of the decoction and tinctura from this plant against *Bacillus diphtheriae* and the antibacterial, anti-inflammation, and antinociceptive activity [3] of the *n*-butanol extract of *Dichondra repens* Forst has been conducted, and the chemical composition of *Dichondra repens* Forst has been reported [4, 5]. But there is not much attention devoted to the essential oil of *Dichondra repens* Forst and its antibacterial activities.

Essential oils have demonstrated numerous biological actions, including antimicrobial activities [6, 7]. These properties are likely due to the many components of these complex mixtures and is facilitated by their ready dilusion across cell membranes.

In this report, the chemical composition and antibacterial activities of the essential oil from *Dichondra repens* Forst is described. The essential oil was obtained from *Dichondra repens* Forst by steam distillation. The amount of components from the essential oil was calculated by the area normalization method. The separated components were identified by GC-MS. The antimicrobial activity of the essential oil has been tested by the method of disc-agar diffusion [8].

A total of 137 compounds were separated and 31 compounds were identified representing about 78.76% of the total oil. The chemical components and relative contents in the essential oil of *Dichondra repens* Forst are given in Table 1. The identified compounds mainly include 6-(methylamino)-phenanthren-3-ol (17.41%), (–)-*trans*-pinane (9.50%), ylangene (8.20%), isocaryophyllene (4.61%), phenol,2,6-dimethoxy- (3.84%), 6-octadecenal (2.88%), 2-methyl-*Z*-4-tetradecene (2.56), 1H-cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene (2.10%).

The disc diameters of inhibition zones, minimum inhibitory concentrations (MIC), and minimum bactericidal concentrations (MBC) of *Dichondra repens* Forst essential oil for the microoganisms tested are shown in Table 2. The data obtained from the disc-agar diffusion method indicated that *Bacillus diphtheriae* had the strongest inhibition zone (23 mm), followed by *β-hemolytic streptococci* (22 mm), *Enterococcus faecium* (20 mm), and *Escherichia coli* (20 mm). *Klebsiella pneumoniae* exhibited a weak inhibition zone (8 mm).

The results of MIC indicated the oil inhibited all microorganisms tested. *Escherichia coli* had the lowest MIC (0.80 μg/mL). The oil was demonstrated to have a strong bactericidal effect. The lowest MBC was 1.50 μL/mL for *Escherichia coli.*

Escherichia coli presented the lowest MIC and MBC values. The strains that presented the biggest inhibition zones (diffusion method) are not always the most sensitive (values of MIC and MBC were lower) because the size of the inhibition zone does not reflect the antibacterial effectiveness of a compound, since it is affected by the solubility of the oil, the diffusion range in the agar, evaporation (it can affect the dose), etc. [9].

Some researchers reported that there is a relationship between the chemical structures of the most abundant compounds in the tested oil and the antimicrobial activity. The essential oils rich in phenolic compounds are widely reported to possess high levels of antimicrobial activity [10–15], which has been confirmed and extended in the present studies. The antimicrobial nature of the essential oil studied is apparently related to its phenolic components. The content of 6-(methylamino)phenanthren-3-ol is the highest in the essential oil of *Dichondra repens* Forst (Table 1). The antimicrobial nature of the essential oil studied is apparently related to the 6-(methylamino)phenanthren-3-ol component.

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TABLE 1. Chemical Components and Relative Contents in the Essential Oil of *Dichondra repens*

Compound	Rt, min	%	Compound	Rt, min	%
2-Methoxyphenol	7.535	0.74	3,7,7-Trimethyl-11-methylene-, (–)-spiro[5.5]undec-2-ene	13.739	1.39
3,7-Dimethyl-1,6-octadien-3-ol	7.728	1.52			
[+]-α-Terpineol	9.228	1.68	(+)-*cis*-Nerolidol	14.074	0.71
trans-Geraniol	9.948	0.44	(–)-Spathulenol	14.388	0.62
2,6-Dimethoxyphenol	11.337	3.84	4-(2,2-Dimethyl-6-methylenecyclohexyl)-2-butanone	14.469	1.66
α-Cubebene	11.803	0.56			
Isocaryophyllene	12.208	4.61	*cis*-9-Tetradecen-1-ol	14.692	0.64
Caryophyllene	12.421	0.69	Bicyclo[3.2.2]non-6-en-3-one	15.097	1.40
β-Humulene	12.786	0.91	*trans*-*Z*-α-Bisabolene epoxide	15.300	0.78
1,13-Tridecanediol, diacetate	12.999	0.41	2,6,6-Trimethyl-(1α,2β,5α)-bicyclo[3.1.1]heptane	15.523	0.97
(–)-*trans*-Pinane	13.100	9.50			
Naphthalene,1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7(1-methenyl)-,[15-(1α,7α,8aα0)-	13.182	0.42	Heptadecane	15.675	0.93
			2-Methyl-*Z*-4-tetradecene	17.216	2.56
1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-	13.242	2.10	Methyl ester hexadecanoic acid, 2-hydroxy-	17.895	1.01
			6-(Methylamino)phenanthren-3-ol	18.007	17.41
Naphthalene,1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7(1-methenyl)-,[1*R*-(1α,7α,8aα0)-	13.313	1.92	6-Octadecenal	18.919	2.88
			cis-4-Hydroxy-3-methylundecanoic acid lactone	19.122	1.70
Ylangene	13.405	8.20			
2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,8a-octahydronaphthalene	13.678	1.93	Phytol	19.852	1.41

TABLE 2. Antibacterial Activities of the Essential Oil from *Dichondra repens*

Bacterias	STD[a] (25 μg/disc)	EO[b] (15 μL/disc)	STD[a] (μg/mL)		EO[b] (μL/mL)	
	Inhibition zone, mm		MIC	MBC	MIC	MBC
Gram Positive						
Bacillus diphtheriae	25	23	0.30	1.50	1.00	2.00
Bacillus subtilis	17	15	1.20	4.00	3.58	7.02
Bacillus cereus	19	19	1.00	3.00	1.58	3.46
Beta-hemolytic streptococci	25	22	0.50	2.00	0.90	2.10
Enterococcus faecium	22	20	0.50	2.00	1.20	2.50
Staphylococcus aureus	20	19	0.50	3.00	2.50	5.32
Gram Negative						
Aeromonas hydrophila	17	11	1.50	4.00	6.20	15.0
Enterobacter aerogenes	20	16	0.22	2.00	1.50	3.00
Enterobacter agglomerans	23	19	0.25	4.00	1.00	2.50
Escherichia coli	18	20	1.00	3.00	0.80	1.50
Klebsiella pneumoniae	16	8	1.00	5.00	8.50	18.0
Salmonella typhi	16	10	0.50	2.00	5.00	12.0

[a]Chloromycetin standard;[b]essential oil.

The antimicrobial activity of the oil is also related to menthol and long-chain alcohols such as 3,7-dimethyl-1,6-octadien-3-ol and [+]-α-terpineol. In fact, long-chain (C_6–C_{10}) alcohols were particularly active against gram-positive bacteria [16], and the antimicrobial properties of alcohols are known to increase with molecular weight [17]. In addition, α-terpineol has been reported to be antibacterial [18].

The oil is a potential source of novel antimicrobial essential oils because of its stronger bactericidal effect, particularly on *Escherichia coli*. Our study suggests that essential oils can be a new medicinal resource for antibacterial agents against *Escherichia coli.*

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