JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2002,** *50,* 3147−3149 **3147**

Essential Oil Constituents and in Vitro Antimicrobial Activity of *Decalepis hamiltonii* Roots against Foodborne Pathogens

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Hydrodistillation of *Decalepis hamiltonii* roots yielded an essential oil (0.33% v/w) that contained 2-hydroxy-4-methoxybenzaldehyde (37.45%), 2-hydroxybenzaldehyde (31.01%), 4-*O*-methylresorcylaldehyde (9.12%), benzyl alcohol (3.16%), and α-atlantone (2.06%) as major constituents, with aromatic aldehydes constituting the main fraction of this root's essential oil. The oil was tested for its antimicrobial activity against foodborne pathogens responsible for food spoilage and human pathologies using standard antimicrobial assays. It exhibited strong antimicrobial activity against *Bacillus cereus*, *Bacillus megaterium*, *Candida albicans*, *Escherichia coli*, *Micrococcus luteus*, *Micrococcus roseus*, and *Staphylococcus aureus* at a concentration range of 1:0 with inhibitory activities of 27, 23, 16, 19, 22, 19, and 23 mm, respectively, which are comparable to those of the standards. The roots of *D. hamiltonii*, therefore, may be considered as an inexpensive source of an essential oil rich in antimicrobial compounds against foodborne pathogens.

KEYWORDS: ***Decalepis hamiltonii*; Asclepiadaceae; roots; essential oil constituents; antimicrobial activity; foodborne pathogens**

INTRODUCTION

The general public perception of food poisoning is that of outbreaks, and cases are increasing at an alarming rate. Several food additives are used to extend food shelf life and inhibit the growth of pathogenic microorganisms. In recent years, there has been an increasing interest in the use of natural substances with questions concerning the safety of synthetic compounds, which have promoted more detailed studies on plant resources. Higher plants and their essential oils, used since antiquity in folk medicine and in preserving foods, provide a wide range of compounds that possess antimicrobial activities.

The use of essential oils in the production of perfumes and beverages is well-known (*1*). Their uses in medicinal drugs and in controlling harmful insects are also quite common (*2*). However, the study of their antimicrobial activity in foodstuffs has received little attention. Identification of such compounds with wide biological activity is critical for mankind as it aids in the search for chemical structures that should help in designing new drugs as therapeutic agents against foodborne pathogens.

Decalepis hamiltonii Wight & Arn. (Asclepiadaceae), an endemic climbing shrub, a native of Peninsular India and distributed in forest areas of the Eastern and Western Ghats, locally known as *nannari* in the Telugu language, finds use as a culinary spice due to its high-priced aromatic roots (*3*, *4*). The roots are now being used in place of *Hemidesmus indicus*, popularly known as Indian sarasaparilla, which are aromatic and used in the Indian traditional system of medicine as a blood purifier, diuretic, antirheumatic, and antidiarrheal agents (*5*). The roots of *D. hamiltonii* are used as a flavoring agent, appetizer, blood purifier, and preservative (*3*). The highly aromatic roots have been exploited by destructive harvesting, which has endangered the survival of this plant in its natural habitats (*6*). It has been found to have bioinsecticidal activity on storage pests at lethal and sublethal levels (*7*). An earlier study has shown that the root of the plant can be used as a fish poison and can be stored for long periods without being affected by microbes (*8*). These highly aromatic roots are used in preparing the drink locally known as nannari, which has a cooling effect in summer, without any toxic effects in humans (*9*).

Although members of Asclepiadaceae are medicinally useful, no attempt has been made to study their essential oil constituents and biological properties (*5*). This prompted us to investigate roots of *D. hamiltonii* for essential oil constituents and antimicrobial activity against different foodborne pathogens using standard microbial assays.

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10.1021/jf011541q CCC: $22.00
Published on Web 04/30/2002

EXPERIMENTAL PROCEDURES

Plant Material. Roots of *D. hamiltonii* were collected from the deciduous forests in Nallamalais of the Kurnool district, Andhra Pradesh, India, in November 2000, and an identified voucher specimen documenting this collection has been deposited in the Sri Krishnadevaraya University (SKU) Herbarium.

Oils Preparation. The roots (600 g), in small pieces, were subjected to hydrodistillation for 8 h, following the *Egyptian Pharmacopoeia* (*10*) method.

Gas Chromatographic (GC) Analyses. A Varian 3300 gas chromatograph equipped with an FID detector and a fused silica capillary column (28 m × 0.25 mm i.d.), coated with bonded phase DB-1 of 0.25 μm film thickness, was used for analysis. Injection of a hexane solution (1 μL) was used, and the chromatogram was produced by holding the oven temperature at 50 °C for 1 min and then programming it from 50 to 270 °C over 40 min. Helium was used as the carrier gas at a linear flow rate of 30 mL s^{-1}, measured at 150 °C, with a split ratio of 1:30, and the septum sweep was held constant at 10 mL min^{-1}. Quantification and retention time determinations were carried out with a Spectra Physics SP 4290 integrator.

Calculation of Kovats Retention Indices (RI). The oil was spiked with a standard mixture of homologous *n*-alkane series (C_8−C_{23}) and analyzed by GC under the above-mentioned conditions. Retention indices were directly obtained by application of Kovats' procedure (*11*, *12*).

Gas Chromatographic—Mass Spectrometric (GC-MS) Analysis. These were obtained with a Hewlett-Packard 6890 series II chromatograph linked to a Hewlett-Packard 5973 mass spectrometer system equipped with an HP automatic injector and a 30 m HP-5 capillary column (0.25 mm i.d.; film thickness = 0.25 μm). The ionization energy was 70 eV. A sample of 1.0 μL of a 2% solution of the oil in *n*-heptane was injected, with a split ratio of 100:1. Identification of the oil components was established using a Wiley MS data library. The program and injector temperatures were the same as used for GC analysis above.

Components Identification. The oil constituents were identified by matching their mass spectral and retention index data with those reported in the literature (*13*−*15*).

Microorganisms and Culture Methods. Potato sucrose broth, Czepeck's broth, and nutrient agar were purchased from Qualigens Fine Chemicals, Mumbai, India; glucose and analytical grade solvents were purchased from SD Fine Chemicals Ltd., Mumbai, India, and antibiotics from Sigma, Poole, U.K. Glass-distilled water was used throughout. A total of 15 microorganisms were used: *Aspergillus fumigatus* (MTCC 1811), *Aspergillus niger* (MTCC 1344), *Bacillus cereus* (MTCC 430), *Bacillus megaterium* (MTCC 428), *Bacillus subtilis* (MTCC 121), *Candida albicans* (MTCC 183), *Candida tropicalis* (MTCC 184), *Escherichia coli* (MTCC 1687), *Fusarium oxysporum* (MTCC 1755), *Micrococcus luteus* (MTCC 1541), *Micrococcus roseus* (MTCC 2522), *Proteus mirabilis* (MTCC 1429), *Proteus vulgaris* (MTCC 1771), *Pseudomonas aeruginosa* (MTCC 1688), and *Staphylococcus aureus* (MTCC 737). All of the microbial strains were purchased from Microbial Type Culture Collection Centre (MTCC), Institute of Microbial Technology, Chandigarh, India. Media were sterilized by autoclaving at 120 °C for 15 min, and all subsequent manipulations were carried out in a laminar flow cabinet.

Antimicrobial Assays. Before experimentation, the oil was diluted with equal volumes of 4% DMSO in H_2O at concentration ranges of 1:0, 1:1, and 1:2. Susceptibility of the test organism to the oil was determined by employing the standard disk diffusion technique (*16*). Whatman No. 1 filter paper disks of 6 mm diameter, placed in dry Petri dishes, were autoclaved. These sterile filter paper No. 1 disks (5−10) were added to each of the test oils and shaken thoroughly. The filter paper disks were allowed to dry and were carefully placed over the spread cultures and incubated at 37 °C for 24 h for bacteria and at 28−30 °C for 48 h for fungi. Paper disks treated with antimicrobial standards alone served as control. The definite zone of inhibition of any dimension surrounding the paper disks was measured accurately to the nearest millimeter by means of a metric ruler and an illuminated colony counter. In all such cases where the zone of inhibition was found to be >10 mm, it was ascertained whether the activity was microbistatic or microbicidal. This was achieved by transferring a loopful of contents from clear zones into sterile broth and incubating them for 3−4 days or more. Turbidity in the tubes indicated microbial activity, whereas a clear broth would show microbicidal activity. Simultaneously different standard antimicrobial compounds were also tested for all microbes under study in similar conditions so as to compare the degree of inhibition exhibited by the essential oils. The oils were subjected to the test of sterility and were found to be free of microorganisms. Each plate carried a blank disk in the center to serve as control, and an average of triple independent readings for each microorganism was recorded.

Table 1. Composition of the Root Essential Oil of *D. hamiltonii*

peak	compound	%	RI	RT[a] (min)	method of identification[b]
1	2-hydroxy-4-methoxybenzaldehyde	37.45	664	11.25	RI, GC-MS
2	β-phellandrene	2.44	840	13.21	RI, GC-MS
3	1-ethyl-3,5-dimethyl benzene	1.50	909	13.37	RI, GC-MS
4	β-pinene	2.01	971	13.72	RI, GC-MS
5	benzyl alcohol	3.16	1005	14.69	RI, GC-MS
6	γ-hexalactone	0.81	1006	15.32	RI, GC-MS
7	2-hydroxybenzaldehyde	31.01	1008	15.40	RI, GC-MS
8	4-*o*-methylresorcylaldehyde	9.12	1032	15.80	RI, GC-MS
9	α-atlantone	2.06	1046	16.58	RI, GC-MS
10	γ-terpinene	1.97	1054	18.58	RI, GC-MS
11	2-phenylethanol	1.03	1180	19.99	RI, GC-MS
12	unknown	1.02	1238	21.28	RI, GC-MS
13	4-methoxybenzaldehyde	0.99	1226	22.47	RI, GC-MS
14	geraniol	1.12	1238	23.58	RI, GC-MS
15	*trans*-anethole	1.14	1264	24.62	RI, GC-MS
16	β-caryophyllene	1.19	1433	25.61	RI, GC-MS
17	*trans*-α-bergamotene	1.04	1434	26.53	RI, GC-MS
18	*trans*-cadinol	0.88	1636	27.40	RI, GC-MS
	identified components	99.94			
	oil yield	0.33			

[a] RT, retention time on an HP-5 (30m) column in minutes. [b] Identification: GC-MS, gas chromaography–mass spectrometry; RI, Kovats index in DB-1.

RESULTS AND DISCUSSION

Hydrodistillation of the roots of *D. hamiltonii* yielded 0.33% (v/w) of oil with a sweet aromatic odor. GC and GC-MS analyses revealed the presence of at least 18 components, 17 of which represented 98.92% of the oil, with major components being 2-hydroxy-4-methoxybenzaldehyde (37.45%), 2-hydroxybenzaldehyde (31.01%), 4-*O*-methylresorcylaldehyde (9.12%), benzyl alcohol (3.1%), β-caryophyllene (1.19%), and α-atlantone (2.06%). The percentage compositions and modes of identification of the oil components are listed in **Table 1**. The oil consisted chiefly of aromatic aldehydes (78.57%), accompanied by relatively much smaller amounts of monoterpene hydrocarbons (6.42%), alcohols (4.19%), and ketones (2.87%). Of the monoterpenes, the principal member was β-phellandrene. Another distinguishing feature was the presence of appreciable amounts of α-atlantone (2.06%), which has insecticidal properties and, being nontoxic to mammals, is used to control household insects. The distilled roots were found to be deficient in oxygenated monoterpenes, oxygenated sesquiterpenes, and sequiterpene hydrocarbons. It is noteworthy that a phenolic component, *trans*-anethole (1.14%), was present in the volatile fraction. β-Pinene (2.01%), which occurs in a noticeable amount, is also used as a disinfectant and insecticide (*17*). It has been previously reported that the roots can be stored for long periods, unaffected by microorganisms, insects, and storage pests (*8*). This may be due to the presence of volatile compounds.

The essential oils of *D. hamiltonii* roots showed strong antimicrobial activity against foodborne pathogens such as *B.*

Table 2. Antimicrobial Activity of the Essential Oil of *D. hamiltonii* Roots against Foodborne Pathogens[a]

microorganism	zone of inhibition (mm)			
	1:0	1:1	1:2	standard[b]
Aspergillus fumigatus MTCC 1811	7	3	–	14
Aspergillus niger MTCC 1344	–	–	–	NT
Bacillus cereus MTCC 430	27	27	21	27
Bacillus megaterium MTCC 428	23	21	13	27
Bacillus subtilis MTCC 121	–	–	–	NT
Candida albicans MTCC 183	16	16	11	12
Candida tropicalis MTCC 184	7	4	–	12
Escherichia coli MTCC 1687	19	19	15	19
Fusarium oxysporum MTCC 1755	17	16	11	12
Micrococcus luteus MTCC 1541	22	21	14	20
Micrococcus roseus MTCC 2522	19	17	16	20
Proteus mirabilis MTCC 1429	5	4	–	11
Proteus vulgaris MTCC 1771	12	12	7	11
Pseudomonas aeruginosa MTCC 1688	11	9	9	13
Staphylococcus aureus MTCC 737	23	22	16	23

[a] Values are mean of three replicates; –, no inhibition; NT, not tested. [b] Clidamycin (2 mg/mL) for *S. aureus*; gentamycin (2 mg/mL) for *Ps. aeruginosa*, *Pr. mirabilis*, and *Pr. vulgaris*; tetracyclin (3 mg/mL) for *B. cereus*, *B. megaterium*, *E. coli*, and *B. subtilis*; clotrimazole (5 mg/mL) for *C. albicans* and *C. tropicalis*; nystatin (10 mg/mL) for *A. fumigatus*, *A. niger*, and *F. oxysporum*; chloramphenicol (100 μg/mL) for *M. luteus* and *M. roseus*.

cereus, *B. megaterium*, *C. albicans*, *E. coli*, *M. luteus*, *M. roseus*, and *S. aureus* at a concentration range of 1:0 with inhibitory activities of 27, 23, 16, 19, 22, 19, and 23 mm, respectively. *B. subtilis* and *A. niger* remain unaffected by the oil. The oil also showed moderate activity against *Ps. aeruginosa* (11 mm) and *Pr. vulgaris* (12 mm) and low inhibitory activities of 7, 7, and 5 mm against *A. fumigatus*, *C. tropicalis*, and *Pr. mirabilis*, respectively. These results could be comparable with the similar effects produced by the standards (**Table 2**). If produced commercially, these oils can be exploited as effective antimicrobials against foodborne pathogens after undergoing successful experimental trials.

D. hamiltonii roots have a strong aromatic odor and volatiles possessing in vitro antimicrobial activity against foodborne pathogens. This antimicrobial activity is due to the presence of a mixture of aldehydes, monoterpenes, sesquiterpenes, and alcohols, which can find application as a preservative for canned and stored foods.

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Received for review November 20, 2001. Revised manuscript received March 12, 2002. Accepted March 14, 2002. We gratefully acknowledge financial support from the Department of Biotechnology, Government of India (Grant BT/PR0691/PB/17/033/97).

JF011541Q