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SHORT COMMUNICATION

CHEMICAL COMPOSITION OF ESSENTIAL OIL OF *ACACIA CRASSICARPA* BENTH. (FABACEAE) FROM VIETNAM

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ABSTRACT. This research aimed to identify the volatile compounds found in the fresh leaves of *Acacia crassicarpa* Benth. This is the first phytochemical investigation of this species. Essential oils from the leaves of *A. crassicarpa* were obtained by hydro-distillation and analyzed by gas chromatography coupled with mass spectrometry (GC/MS). Sixty-one compounds accounting for 95.8% of the leaf oil were identified. The classes of compounds identified in the oil sample were aldehydes (30.7%), sesquiterpene hydrocarbons (25.2%), alkanes (19.1%), oxygenated monoterpenes (3.6%) oxygenated sesquiterpenes (2.3%), monoterpene hydrocarbons (0.8%) and others (14.2%). The major constituents in the leaf oil were tridecanal (24.5%), (E)-caryophyllene (11.7%), n-heneicosane (7.2%), squalene (6.5%), and 7-tetradecenal (5.9%).

KEY WORDS: *Acacia crassicarpa*, Essential oil, Tridecanal, (E)-Caryophyllene

INTRODUCTION

Acacia Mill. is a genus belonging to the subfamily Mimosoideae within the family Fabaceae [1]. There are 1,350 *Acacia* species distributed worldwide, with more than 960 of them native to Australia [2]. *Acacia* is a genus of shrubs and trees that play an important role in ecology and are well adapted to harsh environmental conditions such as low nutrient availability, drought, and salinity [3]. Additionally, numerous *Acacia* species are widely recognized for their utilization as sources of bioactive compounds and traditional medicines, demonstrating a range of pharmacological uses, including hypoglycemic, antibacterial, and anti-inflammatory effects, as well as spasmogenic and vasoconstrictor actions, cytotoxicity, and antioxidant properties [4-10]. However, although there are more than 1350 *Acacia* species, studies on the volatile compounds from the essential oils of *Acacia* species are limited [11-17].

Acacia crassicarpa is a tree that commonly grows up to 15 m tall and has hard grey or grey-brown bark. The phyllodes are 8-27 cm long and 1-4.5 cm wide and are resinous, glabrous, pale green or grey-green. They are normally lanceolate-falcate and broadest below the middle and curved along both margins. The inflorescences are light golden to pale yellow with spikes 2-7 cm long occurring in groups of 2-6 in the axils of branchlet extremities. Seeds are transversely arranged in the pod, black, shiny, and 5-6 mm long [18]. *A. crassicarpa,* native to Queensland, Australia, and southern New Guinea, has demonstrated exceptional growth rates, making it one

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of the most suitable *Acacia* species for afforestation and rehabilitation of degraded sites in seasonally dry tropical regions, particularly in Southeast Asia [3]. *A. crassicarpa* has been planted for multiple purposes, including fuelwood, charcoal, timber, paper production, green manure, and dune stabilization [1, 3, 18-21]. In Vietnam, *A. crassicarpa* has been planted since the 1980s for sand dune protection, as well as for wood and paper production [22]. Especially, in Australia the bark was used traditionally to make baskets and rope by villagers, and young roots were roasted and used as a traditional food source [3]. *A. crassicarpa* was first described in 1842 in Queensland, Australia by George Bentham[23]. Remarkably, despite *A. crassicarpa* being discovered nearly two centuries ago, its medicinal uses and chemical composition and biological activities remain limited [24, 25]. Therefore, the present study investigates, for the first time, the chemical constituents of the essential oil extracted from the leaves of *A. crassicarpa*.

EXPERIMENTAL

Materials and methods

The fresh leaves (2.0 kg) of *A. crassicarpa* were collected from Phong Dien district, Thua Thien Hue province (16°37′19.19"N, 107°23′10.38"E) in August 2023, and identified by Dr. Le Tuan Anh, Viet Nam National Museum of Nature. A voucher specimen (AC-T231) has been deposited in the Faculty of Pharmacy herbarium, Hue University of Medicine and Pharmacy, Hue University, Vietnam.

A **B**

Figure 1. A: The whole *A. crassicarpa* in its natural habitat and B: leaf and inflorescence of *A. crassicarpa.*

Hydrodistillation of the essential oils

Before the hydrodistillation process, the fresh leaves were cleaned and shredded in accordance with the methods utilized by previous studies [26, 27]. The oils were obtained via hydrodistillation using a Clevenger apparatus for 4 hours at normal pressure, according to the Vietnamese Pharmacopoeia [28]. The essential oils were dried by sodium sulfate (Na_2SO_4) and then stored in a vial at 4 ºC. The experiments were repeated in triplicate.

GCMS analysis

The chemical composition of the essential oil was analyzed using the GCMS-QP2010 Plus system, manufactured by Shimadzu in Kyoto, Japan. The system was equipped with an Equity-5

capillary column measuring 30 m in length and 0.25 mm in diameter, coated with a 0.25-micrometer-thick film and coupled with a mass spectrometer (MSD QP2010 Plus) for GC/MS analysis. To carry out the analysis, the oil was first diluted with n-hexane at a ratio of 1:100, and 1 μL of the diluted oil was used. Helium gas was used as the carrier gas at a flow rate of 1.5 mL/min, while the injector and interface temperatures were set at 280 °C. The column temperature was programmed to increase from 60 °C (2 min hold) to 240 °C at a rate of 3 °C/min (10 min hold), followed by an increase to 280 °C at a rate of 5 °C/min (40 min hold). The samples were injected in splitless mode and split mode. The mass spectrometer was set to an ionization voltage of 70 eV and an acquisition scan mass range of 45-500 (m/z) at a sampling rate of 1.0 scan/s [26, 27].

Identification chemical composition

The individual constituents present in the essential oil were determined by comparison of the mass spectral data with MS fragmentation patterns of known compounds in literature as described recently [29, 30]. The retention indices (RI Exp.) of each compound were also compared to a homologous series of n-alkanes (C_8-C_{33}), run under identical experimental GC conditions as with sample [30]. Also, co-injection with standard compounds under the same GC conditions was used to identify compounds. Quantification was done by using the relative peak area percentage.

RESULTS AND DISCUSSION

The average yield of leaf oils of *A. crassicarpa* was 0.03 ± 0.01% fresh weight (w/w). The essential oils of leaves were a pale, light yellow liquid with a characteristic aromatic odor. The oils were lighter than water. Compared with previous findings, the yield of leaf oil from *A. gerrardii* (0.04%) [17] was similar to that of *A. crassicarpa* in our study. In contrast, the yield of leaf oil from *A. tortilis* (0.12%) [12] was significantly higher than that of *A. crassicarpa*. There were sixty-one compounds accounting for 95.8% (Table 1) of the compounds detected in the leaves oils of *A. crassicarpa*. This analysis revealed that the classes of compounds were aldehyde (30.7%), serquiterpene hydrocarbon (25.2%), alkanes (19.1%), oxygenated monoterpenes (3.6%) oxygenated serquiterpenes (2.3%), monoterpene hydrocarbon (0.8%) and others (14.2%) (including triterpen (6.5%), sesquiterpenoid ketone (2.2%), alkene (1.5%), diterpen (1.2%), acyclic monoterpenoid ketone (1%), ionones (0.6%), fatty alcohols (0.6%), sesquiterpenoid ester (0.4%) and diterpenoid ester (0.2%)). The principal compounds found in the leaf oil were tridecanal (24.5%), (E)-caryophyllene (11.7%), n-heneicosane (7.2%), squalene (6.5%), and 7-tetradecenal (5.9%). Our findings indicated several differences in oil constituents compared to *Acacia* species oils studied in the previous works [11-17]. For example, analysis of essential oil from the stem bark of *A. nilotica* and *A. albida* obtained in Nigeria showed that the major compounds were menthol (34.9%), limonene (15.3%) of *A. nilotica* [13] and α-pinene (18.6%) of *A. albida* [13]. In another study in Tunisia the author analyzed five different parts of *A. cyanophylla* including the leaf, the root, the stem, the flower, and the pod [14]. The study found that the major components in leaf oil were dodecanoic acid (22.8%), hexahydrofarnesyl acetone (12.1%) and benzyl benzoate (11.5%), while tridecanal accounted for 1.1% and (E)-caryophyllene was absent. In comparison, our analysis found larger quantities of tridecanal (24.5%) and (E)-caryophyllene (11.7%) respectively. The dominant constituents of the root were phenylethyl salicylate (34.9%), benzyl benzoate (32.4%), and 2-phenylethyl benzoate (19.4%). The stem primarily contained heptyl valerate (17.3%), tetradecanoic acid (15.0%), benzyl butyrate (14.2%), dodecanoic acid (9.4%). The flower contained dodecanoic acid (66.5%), hexahydrofarnesyl acetone (8.4%) and the pod primarily contained nonadecane (36.0%) and hexahydrofarnesyl acetone (23.3%) [14]. The essential oils from flowers of *Acacia* species from Argentina were p-anisaldehyde (14.4%), (E,E)-farnesyl acetate (14.0%), eugenol (11.2%), benzyl alcohol (11.0%),

methyl salicylate (8.1%) in *A. caven.* while essential oils from *A. aroma* were methyl salicylate (42.0%), eugenol (15.5%), cuminyl alcohol (9.1%), and (E)-anethole (8.9%) [31]. A recent study of the leaf oil of *A. gerrardii* from Saudi Arabia found that pulegone (35.11 %), carvacrol (27.36 %), and neo-dihydrocarveol (4.67%) were the major components [17]. These findings show that there can be significant differences in chemical composition between *Acacia* species. These differences may be due to ecological and climatic variations, sample collection times, or analytical and identification methods [12, 32, 33].

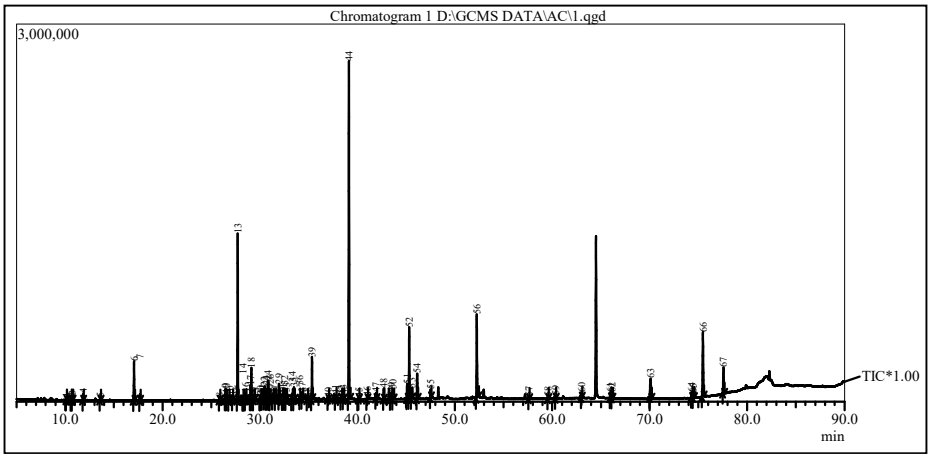

Figure 2. Chromatogram (GC) of leaves essential oil from *A. crassicarpa.*

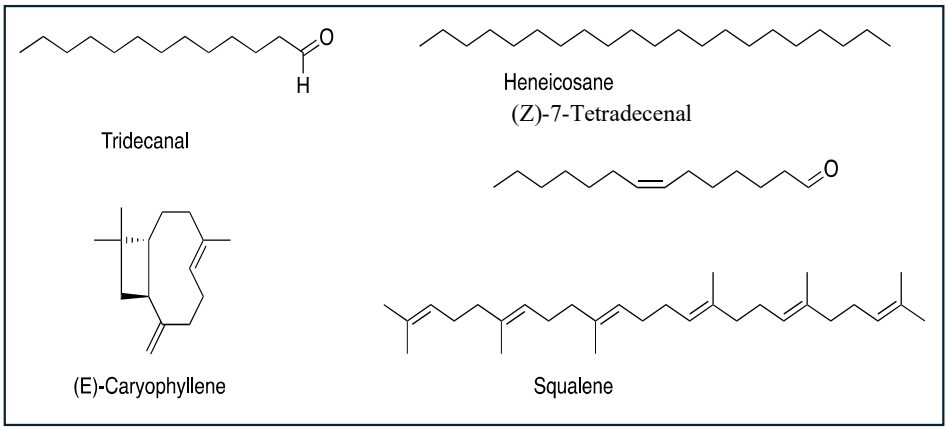

Figure 3. Chemical structure of the major compounds identified in the essential oil of *A. crassicarpa* leaves.

Table 1. Chemical composition of the essential oil from the leaves of *A. Crassicarpa.*

No	RT	Compounds[a]	RI[b]	RI[c]	Concentration
1	10.13	α-Terpinene	1014	1016	0.2
2	10.62	β-Phellandrene	1025	1028	0.2
3	10.75	1,8-Cineole	1026	1031	0.3
4	11.85	γ-Terpinene	1054	1057	0.4
5	13.60	Tetrahydro-Linalool	1098	1100	0.2
6	17.07	Terpinen-4-ol	1174	1177	2.6
7	17.68	α-Terpineol	1186	1191	0.4
8	25.85	α-Ylangene	1373	1377	0.2
9	26.47	1-Tetradecene	1388	1391	0.8
10	26.53	β-Elemene	1389	1393	0.5
11	26.80	n-Tetradecane	1400	1399	0.2
12	27.18	(Z)-Caryophyllene	1408	1408	0.4
13	27.71	(E)-Caryophyllene	1417	1421	11.7*
14	28.27	(E)-α-Ionone	1428	1435	0.2
15	28.35	trans-α-Bergamotene	1432	1437	0.2
16	28.51	Aromadendrene	1439	1441	0.6
17	29.03	Geranyl acetone	1453	1453	1.0
18	29.12	trans-Prenyl limonene	1457	1455	2.3
19	29.41	allo-Aromadendrene	1458	1462	0.2
20	30.05	γ-Muurolene	1478	1478	0.4
21	30.25	Germacrene D	1480	1483	0.4
22	30.46	β-Selinene	1489	1488	1.1
23	30.83	δ-Selinene	1492	1497	1.7
24	30.83	α-Selinene	1498	1497	1.7
25	30.93	n-Pentadecane	1500	1499	0.4
26	31.03	α-Muurolene	1500	1502	0.2
27	31.31	(E,E)-α-Farnesene	1503	1509	0.6
28	31.58	γ-Cadinene	1513	1516	0.2
29	31.95	δ-Cadinene	1522	1525	1.2
30	32.30	trans-Cadina-1,4-diene	1533	1534	0.2
31	32.42	β-(E)-Ionol acetate	1537	1537	0.4
32	32.52	α-Cadinene	1537	1540	0.2
33	32.68	Selina-3,7(11)-diene	1544	1544	0.6
34	33.29	Germacrene B	1559	1559	0.5
35	33.49	(E)-Nerolidol	1561	1564	0.9
36	34.09	Spathulenol	1577	1580	0.2
37	34.32	Caryophyllene oxide	1582	1586	0.7
38	34.86	n-Hexadecane	1600	1599	0.2
39	35.34	Tetradecanal	1611	1612	3.1
40	37.02	α-Cadinol	1652	1657	0.3
41	37.76	n-Tetradecanol	1671	1677	0.4
42	38.56	n-Heptadecane	1700	1699	0.4
43	39.13	Tridecanal	-	1714	24.5
44	40.15	(2E,6E)-Farnesal	1740	1743	0.2
45	41.02	n-Pentadecanol	1773	1768	0.2
46	41.91	1-Octadecene	1789	1793	0.6
47	43.50	(2E,6E)-Farnesyl acetate	1845	1839	0.4
48	45.11	Cubitene	1878	1887	1.2
49	45.32	(Z)-7-Tetradecenal	-	1893	5.9
50	45.58	n-Nonadecane	1900	1901	0.9
51	46.14	(5E,9E)-Farnesyl acetone	1913	1918	2.2

No	RT	Compounds[a]	RI[b]	RI[c]	Concentration
52	52.25	n-Heneicosane	2100	2113	7.2
53	57.62	n-Tricosane	2300	2298	0.2
54	59.57	Methyl labdanolate	2381	2370	0.2
55	60.34	n-Tetracosane	2400	2398	0.3
56	63.01	n-Pentacosane	2500	2498	0.6
57	66.14	Hexacosane	2600	2597	0.7
58	70.09	Heptacosane	2700	2698	2.6
59	74.49	Octacosane	2800	2798	0.6
60	75.47	Squalene	2831	2829	6.5
61	77.58	Nonacosane	2900	2897	2.3
Monoterpene hydrocarbon					0.8
Oxygenated monoterpene					3.6
Sesquiterpene hydrocarbon					25.2
Oxygenated sesquiterpene					2.3
Alkanes					19.1
Aldehyde					30.7
Others					14.2
Total					95.8

[a]Elution order on Equity-5 column, [b]Retention indices on Equity-5 column, [c]Literature retention indeices [30], *co-injection with an authentic sample, - Not identified.

CONCLUSION

The goal of this work is to provide a unique contribution to the literature by reporting, for the first time, the chemical constituents of the essential oil from the leaves of *A. crassicarpa* planted in Vietnam. The chemical components of the leaf oil mainly consist of aldehydes (30.7%), sesquiterpene hydrocarbons (25.2%), and alkanes (19.1%), respectively. The principal constituents of the leaf oil of *A. crassicarpa* are tridecanal (24.5%), (E)-caryophyllene (11.7%), n-heneicosane (7.2%), squalene (6.5%), and (Z)-7-tetradecenal (5.9%). The new findings of this research will contribute to further studies regarding the chemical composition and bioactivity of *A. crassicarpa.*

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Conflict of interest

There was no conflict of interest declared by the authors.

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