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Chemical Profiling Revealed a Dominant Compound *trans*-Anethole and Biological Evaluation of an Edible Plant *Clausena harmandiana* Containing Essential Oil

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Abstract: This study aimed to characterize the chemical and biological properties of the edible plant, *Clausena harmandiana* (Pierre) Guillaumin. Fresh leaves of this plant were hydrodistilled to obtain the essential oil, which was characterized by GC-MS. Total phenolic content was evaluated by Folin-Ciocalteau assay. Biological activity was assessed by measuring antioxidants, antimicrobial activities, and the inhibitory effects on tyrosinase, collagenase, and α-glucosidase. The primary components were *trans*-anethole (91.44%), followed by estragole (2.98%) and *cis*-anethole (2.55%). The essential oil contained the amount of phenolic 20.63 mg GAE/g extract and showed the activity to scavenge DPPH (IC_{50} 18.48 mg/mL), ABTS (IC_{50} 3.97 mg/mL) radicals and also presented the ability to reduce Fe^{3+}-TPTZ complex on FRAP assay (3.74 mg TEAC/g extract). Additionally, the *C. harmandiana* essential oil exhibited antimicrobial activity against *Candida albicans* (MIC 6.63 mg/ml, MFC 13.25 mg/mL) and interestingly presented enzymatic inhibitory effects on collagenase (IC_{50} 0.059 mg/mL) and α-glucosidase (IC_{50} 0.677 mg/mL). The remarkable activity of inhibition on α-glucosidase was two times more to acarbose (IC_{50} 1.26 mg/mL).

Keywords: *Clausena harmandiana;* Rutaceae; chemical profile; biological activity.

1. Introduction

Clausena harmandiana (Pierre) Guillaumin is an aromatic plant of the Rutaceae family, members of which are characterized by the presence of complex essential oils in schizolysigenous pockets [1]. Some species contained leaves carrying pellucid dots filled with volatile oils. *Clausena harmandiana* is known in Thailand as Prong-Fa, Song Fa, and Song Fa Dong. Its roots provide a

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traditional medicine for headache, colic, and bronchitis, and the leaves are used as a carminative [2]. The young leaves are also used as an edible plant in local cuisines. Additionally, local wisdom in northern Thailand holds that tea made from dried leaves is useful for treating smoking addiction.

A prior study of *C. harmandiana* roots identified 10 coumarin compounds, a ferulate, and 8 carbazoles [2]. Previous research reported only the chemical constituents and biological effects of the root, root bark, and the dried leaf of this plant, while the fresh leaf containing the volatiles were not reported to the best of our knowledge. Therefore, this research aimed to investigate the chemical composition, phenolic content, and antioxidant, antimicrobial, and enzymatic inhibitory effects of *C. harmandiana* essential oil extracted from fresh leaves.

2. Materials and Methods

2.1. Plant Material

Clausena harmandiana (Pierre) Guillaumin was collected in October 2018 from Amphoe Ban Hong, Lamphun province, Thailand. Specimen No. Nichakan-005 was identified by a taxonomist and deposited at Herbarium of Faculty of Pharmacy Chiang Mai University.

2.2. Essential oil Extraction

The fresh leaves of *C. harmandiana* were distilled by hydrodistillation for 1.5 h to obtain the essential oil. Anhydrous sodium sulfate was added to absorb the water from the oil. The essential oil was then transferred into a brown container and stored at 4°C until further investigation.

2.3. Chemical Identification of Essential Oil

The chemical composition of *C. harmandiana* essential oil was identified by Gas Chromatography Mass Spectrometry (GC-MS) using a GC Model TRACE 1300 with TriPlus RSH Autosampler and Triple Quadrupole Mass Spectrometer Model TSQ 8000 Evo (Thermo Scientific). The capillary column TR5-MS, 5% Phenyl methyl polysiloxane; 30 m × 0.25 mm ID, 0.25 µm film thickness (Thermo Fisher) was used for separation. Helium was used as a carrier gas. The auto-injector port was preset at 300°C then 1 µL of the sample was injected with a splitting ratio of 1:20. The helium gas was set at a flow rate of 1.00 mL/min. The program was initiated at 60°C and increased 5°C/min to 90°C for 6 min, followed by 1°C/min to 95°C for 5 min, then 5°C/min to 180°C for 17 min and 1°C/min to 185°C for 5 min, and finally 10°C/min to 250°C for 6 min with a 10 min hold. The NIST Mass Spectral Search Program Version 2.3, May 4 2017 (NIST) and Wiley Registry of Mass Spectral Database (Wiley) were used for chemical matching. The data also were compared with the mass spectra and retention indices (RI) with a series of n-alkane (C_8-C_{40}). The formula below was used to calculate RI [3]. Besides, the retention indices data source on the non-polar column from the NIST chemistry webbook, SRD 69 (www.webbook.nist.gov) and previously reported literature were used for references.

$$RI = 100\ [(t_x\text{-}t_n)/(t_{n+1}\text{-}t_n)+n]$$

In this formula, t_x is the retention time of the analyzed compound, t_n and t_{n+1} are the retention times of n-alkane leaving the chromatographic column before and after the compound under consideration.

2.4. Determination of Phenolic Content

The Folin-Ciocalteau assay [4] was used with some modification to evaluate the total phenolic content of the essential oil. The initial concentration of the sample was prepared at 100 mg/mL in ethanol. The Folin-Ciocalteau reagent was diluted with water (1:10) before testing. The sample (50 µL) was pipetted and mixed with 100 µL Folin-Ciocalteau reagent in a 96-well plate and sodium

carbonate 7.5% w/v (100 µL) was added. The sample mixture was incubated in the dark at room temperature for 30 min. Absorbance was measured at 750 nm. Total phenolic content was calculated and compared with the standard curve of gallic acid standard. The result was reported in terms of gallic acid equivalents (mg GAE/g extract).

2.5. Determination of Antioxidant Activity

2.5.1. DPPH Radical Scavenging Assay

DPPH (2,2-Diphenyl-l-picrylhydrazyl) solution was used to evaluate the radical scavenging capacity of the essential oil according to Adebiyi [5] and Brem [6] with modifications. The radical solution was prepared by using DPPH dissolved in ethanol and absorbance was measured at 520 nm (0.7 ±0.2) before testing. The sample stock solution was dissolved in ethanol, then 20 µL of the sample was pipetted and mixed with 180 µL DPPH solution in a 96-well plate. The mixture was incubated in the dark at room temperature for 30 min, and absorbance was measured at 520 nm. The percent inhibition was calculated according to the formula below. The assay used the Trolox standard for comparison.

$$\% \text{ Inhibition} = [(A_0 - A_1)/A_0] \times 100$$

A_0 is the absorbance of DPPH mixed with the solvent that was used to dissolve sample or standard. A_1 is the absorbance of DPPH mixed with the tested sample or standard.

2.5.2. ABTS Radical Scavenging Assay

The essential oil was evaluated by ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay according to Adebiyi [5] and Re [7] with some modifications. ABTS radical solution was prepared with 7 mM ABTS solution mixed 1:1 with 4.9 mM potassium persulfate ($K_2S_2O_8$) and incubated in the dark at room temperature for 16 h. The solution was diluted with ethanol (1:50), and absorbance was measured at 734 nm. The absorbance of ABTS before testing is 0.7 ±0.2. The stock sample solution was prepared similar to the DPPH assay. Then, 20 µL of sample solution was pipetted and transferred into microplate 96-well. The ABTS radical solution (180 µL) was mixed and incubated in the dark at room temperature for 6 min. The absorbance was measured at 734 nm and Trolox was used as the comparative standard. The percent inhibition was calculated as the formula described above.

2.5.3. Ferric Reducing Antioxidant Power (FRAP) Assay

TPTZ (2,4,6-Tri(2-pyridyl)-s-triazine) was used to evaluate the FRAP value of the essential oil according to Benzie and Strain [8] with modifications. FRAP reagent was freshly prepared with 300 mM acetate buffer, pH 3.6, and 10 mM TPTZ in hydrochloric acid and 20 mM ferric chloride hexahydrate (10:1:1). The sample solution was dissolved in ethanol, and 50 µL were mixed with 150 µL FRAP reagent in a 96-well plate and incubated for 8 min in the dark at room temperature. Absorbance was measured at 600 nm. The results were calculated and compared with the Trolox standard curve and reported as Trolox equivalent antioxidant capacity (mg TEAC/g extract).

2.6. Determination of Antimicrobial Activity

Antimicrobial activity against *Staphylococcus aureus* TBCR4930 and *Candida albicans* TBRC209 was assessed according to Wiegand [9] and CLSI [10]. Colonies of *S. aureus* and *C. albicans* were maintained on Trypticase soy broth (TSB) and Sabouraud Dextrose Broth (SDB) culture media and incubated at 37°C for 4 h. The cultures were suspended in TSB and SDB (compared with McFarland No.0.5) at $1x10^6$ CFU/mL for bacteria and $1x10^4$ spores/mL for fungi. The stock sample solution was prepared in DMSO and diluted with culture medium before use. Culture media (500 µL) were added to a 24-well plate. Then, 500 µL sample solution was added to the first well and

a two-fold serial dilution series was performed, with 500 µL removed from the final well. The stock bacterial and fungal suspensions (500 µL) were added to each well and incubated at 37°C for 24 h. The minimum inhibitory concentration (MIC) was determined as 99.9% transparency of the mixture in turbidity testing. The minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were assessed by subculturing the macroscopically clear mixture from the MIC well on solid media. The MBC and MFC were the concentrations at which 99.9% of microorganisms were killed. Gentamycin and ketoconazole were used as positive control, and DMSO was used as the negative control

2.7. Determination of Enzymatic Inhibitory Activity

2.7.1. Tyrosinase Inhibitor Assay

The essential oil was evaluated for anti-tyrosinase activity according to Dej-adisai [11] and Piao [12] with modification. The test substrate was 0.85 mM L-DOPA (3,4-Dihydroxy-L-phenylalanine ethyl ester) solution. The enzymatic solution (500 U/mL) was freshly prepared using tyrosinase enzymes from mushrooms dissolved in 20 mM phosphate buffer, pH 6.8. The sample was dissolved with a small amount of ethanol and diluted with phosphate buffer, then 20 µL of sample solution was mixed with 20 µL tyrosinase and 140 µL phosphate buffer and incubated at 37°C for 10 min. The substrate solution (20 µL) was added and incubated at 37°C for 20 min. Absorbance was measured at 492 nm. Inhibition was calculated according to the formula below and compared with the standard curve of kojic acid.

$$\% \text{ Inhibition} = 100\times (A_{ct-}[A_{te}-A_{tb}) / A_{ct}$$

A_{ct} is the absorbance of the solvent used to dissolve the sample or standard, mixed with tyrosinase enzyme and substrate. A_{te} is the absorbance of the tested sample or standard mixed with tyrosinase enzyme and substrate. A_{tb} is the absorbance of the tested sample blank or standard mixed with substrate.

2.7.2. Collagenase Inhibitor Assay

Collagenase inhibition was measured according to Thring [13] with some modification. FALGPA (1 mM, *N*-[3-(2-furyl) acryloyl]-Leu-Gly-Pro-Ala) substrate was dissolved in 50 mM tricine buffer, pH 7.4. The collagenase from *Clostridium histolyticum* stock solution (1 U/mL) was freshly prepared at 2-8 °C and dissolved with 18.2 MΩ ultrapure water. The sample was dissolved in ethanol and diluted with tricine buffer, then 20 µL sample solution was pipetted and mixed with 10 µL collagenase enzyme and 30 µL tricine buffer. The mixture was incubated for 20 min at 37°C. The FALGPA substrate solution (40 µL) was added and incubated again at the same temperature for 30 min, and absorbance was measured at 340 nm. The percent inhibition was calculated as described above and compared with an epigallocatechin gallate (EGCG) standard curve.

2.7.3 α-Glucosidase Inhibitor Assay

Anti-α-glucosidase activity was determined according to Kim [14]. A 5 mM solution of PNP-α-D-Glu (4-nitrophenyl α-D-glucopyranoside) in 0.1 M phosphate buffer, pH 6.8, was used as substrate. α-Glucosidase from *Saccharomyces cerevisiae* (1 U/mL) was prepared in the same phosphate buffer. The sample solution was assessed in a manner similar to the tyrosinase inhibition assay. The sample (50 µL) was mixed with 50 µL α-glucosidase solution and phosphate buffer in a 96-well plate and incubated at 37°C for 20 min. Then 50 µL of PNP-α-D-Glu was added and incubated at 37°C for another 20 min. The reaction was stopped with 50 µL of 0.2 M sodium carbonate ($NaCO_3$), and absorbance was measured at 405 nm. The percentage of α-glucosidase inhibitory activity was calculated according to the formula below. Acarbose was used as a standard for comparison.

$$\% \text{ Inhibition} = [A_c-(A_{te}–A_{tb})/A_c] \times 100$$

A_c is the absorbance of the solvent used to dissolve the sample or standard, mixed with α-glucosidase enzyme and substrate. A_{te} is the absorbance of the tested sample or standard mixed with α-glucosidase and substrate. A_{tb} is the absorbance of the tested sample or standard mixed substrate.

2.8 Statistical Analysis

All experiments were performed in triplicate. Data are reported as the mean ± SD. ANOVA and LSD tests for multiple comparisons were used to analyze the data, with statistical significance defined as $p < 0.05$.

3. Results and Discussion

3.1. Chemistry

The percentage yield of the essential oil from fresh leaves of *C. harmandiana* was 1.81%. The physical properties included a characteristic odor, transparent liquid that solidified upon refrigeration. The chemical profile was identified by using the GC-MS technique. The results of their chemical compositions and total ion chromatogram are shown in Table 1 and Figure 1.

The relative abundance of chemical members in the essential oil comprised the major compound groups of phenylpropenes (97.02%), monoterpenes (0.35%) and sesquiterpenes (2.3%). The primary component was *trans*-anethole (91.44%), followed by estragole (2.98%) and *cis*-anethole (2.55%) phenylpropanoids. This result exhibited the percentage relative abundance of a major component different from previous research. Tanruean reported that the essential oil extracted from the dried leave of *C. harmandiana* showed anethole (46.09%) as majority composition followed by camphene (9.61%), β-terpinene (7.87%), *D*-limonene (7.07%), γ-terpinene (3.57%) and 4-terpineol (3.34%), respectively [15]. A comparison of our results and earlier research clearly reveals a substantial difference in the amount and chemical composition of essential oil obtained from fresh versus dried leaves. The phenylpropanoid anethole has been detected in the essential oils of *C. anisum-olens* (Blanco) Merr, *C. heptaphylla* W&A, and *C. Anissata* [16]. Estragole was reported as a major component of *C. Anistata* [16, 17], and other species of *Clausena* carry monoterpenes (terpinolene 16.7% and δ-3-carene 11.6%) and oxygenated monoterpenes (ρ-cymen-8-ol 7.8%), including phenylpropanoids (myristicin 35.3%) [16] as a major compounds have been reported.

Anethole is an aromatic compound found in the Apiaceae, Myrtaceae, Fabaceae, and Illiciaceae families [18] and is a well-known component of anise (*Pimpinella anisum*) and fennel (*Foeniculum vulgare*) [19]. Anethole has a characteristic sweet taste and solidifies to a crystalline form at about 21°C and melts at 22°C [19]. It is used as a flavoring agent and has carminative properties [19], consistent with its use in folk medicine to relieve flatulence. Other traditional uses for anethole include antispasmodic, secretolytic, and antibacterial activities; some practitioners of folk medicine use anise (which contains 80-90% anethole) as an expectorant, to increase productive cough, to increase milk flow in lactating mothers, to relieve menstrual discomfort, and for the treatment of symptoms of male menopause [18]. Anethole also provides antioxidant, antimicrobial, antinociceptive and anti-inflammatory, gastroprotective, anthelmintic, and insecticidal activities [18]. It is used as a flavor additive in many alcoholic drinks due to its distinctly sweet character [18] and in products such as mouthwash, toothpaste, and toilet soap [19].

Furthermore, an essential oil obtained by steam distillation of dried *C. harmandiana* leaves contained 46.09% anethole and phenolic content of 7.07 mg GAE/g extract showed antioxidant activities in DPPH (IC_{50} 2865.26 µg/mL), ABTS (IC_{50} 12.83 µg/mL), and FRAP value (5.37 mg

GAE/g extract) [15]. Besides, 10 coumarin compounds, a ferulate, and 8 carbazoles were identified from *C. harmandiana* roots [2]. Heptazoline showed strong cytotoxic activity on NCI-H187 and methoxymukonal exhibited strong cytotoxicity against MCF-7 and KB cell lines [2]. Moreover, 7-hydroxyheptaphylline and nordentatin were separated from the root of *C. harmandiana* exhibited strong antioxidant activity on lipid peroxidation (IC_{50} 2.95 and 2.90 µM), radical scavenging activity (DPPH, IC_{50} 56.82 and 29.30 µM) and toxicity in KKU-OCA 17 (IC_{50} 88.7 and 46.1 µM) and KKU-124 (IC_{50} 43.7 and 39.1 µM) cultures [20]. In 2013 *C. harmandiana* root also contains a novel carbazole alkaloid, clauraila E [21]. Carbazole alkaloids and coumarins isolated from the root bark exhibited antioxidant, cytotoxic, and apoptotic activities in various cell lines [22].

Table 1. Chemical composition of *C. harmandiana* essential oil.

Compound	**Exp.-RI[a]**	**Ref-RI[b]**	**Ref-RI[c]**	**%Relative abundance (Average ± SD)**
cis-3-Hexen-1-ol	859	857	851-858	0.02 ± 0.00
Sabinene	979	974	954-977	0.03 ± 0.00
cis-3-Hexenyl-1-acetate	1008	1005	1005-1009	0.02 ± 0.00
cis-β-Ocimene	1034	1038	1027-1044	0.14 ± 0.01
trans-β-Ocimene	1045	1049	1032-1054	0.10 ± 0.01
Linalool	1106	1099	1080-1104	0.08 ± 0.01
2-Octyl acetate	1108	1115	-	0.04 ± 0.00
Nonanal	1113	1112	1089-1110	0.01 ± 0.00
Estragole	1211	1196	1195-1203	2.98 ± 0.01
cis-Anethole	1267	1254	1245-1262	2.55 ± 0.07
p-Anisaldehyde	1277	1251	1251-1277	0.05 ± 0.01
Unknown	1297	-	-	0.12 ± 0.02
trans-Anethole	1304	1286	1283-1301	91.44 ± 0.31
β-Elemene	1396	1386	1362-1410	0.06 ± 0.02
trans-Caryophyllene	1431	1419	1410-1451	0.08 ± 0.01
α-Humulene	1468	1454	1424-1488	0.03 ± 0.00
allo-Armadendrene	1472	1461	1442-1474	0.02 ± 0.00
α-Curcumene	1489	1488	1479-1493	0.03 ± 0.01
Germacrene-D	1493	1482	1453-1519	0.24 ± 0.01
α-Zingiberene	1502	1500	1474-1509	0.27 ± 0.02
Bicyclogermacrene	1508	1507	1484-1532	1.07 ± 0.06
δ-Cadinene	1529	1522	1486-1563	0.09 ± 0.01
Unknown	1592	-	-	0.04 ± 0.01
Spathulenol	1594	1582	1548-1622	0.24 ± 0.03
Aromadendrane-4,1-diol	1603	*ND	-	0.06 ± 0.00
Unknown	1652	-	-	0.03 ± 0.01
τ-Cadinol	1660	1640	1635-1665[d]	0.05 ± 0.01
α-Cadinol	1675	1653	1632-1673[d]	0.06 ± 0.01
Unknown	1681	-	-	0.02 ± 0.01

t_R: Retention time, Exp.-RI[a]: Retention indices from analysis, Ref.-RI[b]: Retention indices from the references with similar column analysis, Ref.-RI[c]: Retention indices data references on non-polar column from NIST chemistry webbook (www.webbook.nist.gov) [23], [d][24], *ND: Not detected.

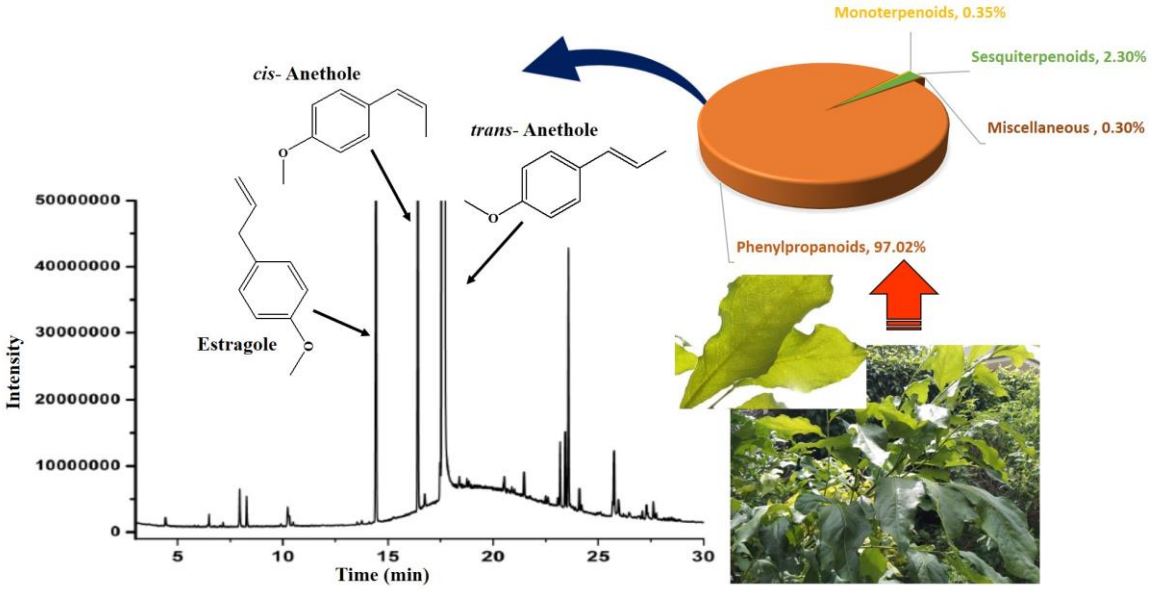

Figure 1. Total ion chromatogram and the compound groups calculated from the relative abundance of chemical composition of *Clausena harmandiana* essential oil

3.2. Phenolic Content and Antioxidant Activity

The essential oil was assessed for total phenolic content and antioxidant activity. The phenolic compounds are a group of small molecules characterized by their structures having at least one phenol unit (C_6H_5OH) [25]. In this assay showed phenolic content 20.63 mg GEA/g extract by using Folin-Ciocalteau method. This protocol involved the oxidation of phenols in alkaline solution [26]. The phenol structure in the molecule of the chemical composition from the essential oil will react with Folin-Ciocalteau reagent (which contains phosphomolybdic/phosphotungstic acid complexes) changed from yellow color to a blue complex. Therefore, the amount of phenolic content of the essential oil probably estimated from major chemical compounds (*trans*-anethole, estragole and *cis*-anethole) that contained phenolic ether structure in their molecules. Besides, the essential oil exhibited the ability to reduce Fe^{3+}-TPTZ to Fe^{2+}-TPTZ complexes in FRAP assay equivalent to Trolox standard was 3.74 mg TEAC/g extract. We also observed free radical scavenging activity in ABTS (IC_{50} 3.97 mg/mL) and DPPH (IC_{50} 18.48 mg/mL) assays. The results are shown in Table 2.

Our observations showed a greater phenolic content than prior reports, which showed 7.07 mg GAE/g extract in the essential oil extracted by steam distillation of dried *C. harmandiana* leaves [15]. Consideration the factors especially the process of sample preparing before extraction including the procedure of extraction and other such as the plant collection, the season of harvesting were affected the essential chemical compounds in plants which related to biological activity. The phenolic compounds found in this essential oil referred to the major component, *trans*-anethole, estragole and *cis*-anethole, which consisted of phenolic ethers structure [19] including common structure of phenol in the chemical composition of the essential oil. The antioxidant activity of *C. harmandiana* essential oil is attributable to the phenylpropanoids (*trans*-anethole, estragole, and *cis*-anethole) and mono- and sesquiterpenes in this essential oil. The potential of antioxidant activity depends on the well-established chemical constituents, phenolic contents, as well as secondary metabolites with conjugated double bonds [19, 27]. However, *trans*-anethole was reported as a remarkable compound in anise and fennel oils, it also showed antioxidant activity [28]. The mechanism to reaction with free radicals was described at the phenyl side chain that conjugated with the aromatic ring of *trans*-anethole structure can simply be conjugated with radical cation, which leads to delocalization of the electron in the aromatic ring and also to stabilization at 1,4-interaction of the methoxy group [28, 29].

Table 2. Total phenolic content and antioxidant activity of *C. harmandiana* essential oil

Sample	Total phenolic mg GAE/g extract	Antioxidant activity		
		DPPH (IC_{50} mg/mL)	ABTS (IC_{50} mg/mL)	FRAP (mg TEAC/g extract)
ECH	20.63 ± 0.94	18.48 ± 0.37	3.97 ± 0.46	3.74 ± 0.06
Trolox	-	8.63±0.10	4.57±0.77	-

ECH: essential oil, -: not tested

3.3. Antimicrobial and Enzymatic Inhibitory Activities

Data regarding the essential oil's antimicrobial activity and its inhibition of tyrosinase, collagenase, and α-glucosidase are shown in Table 3. The essential oil inhibited yeast *C. albicans* with an MIC of 6.63 mg/mL and MFC of 13.25 mg/mL, while its effect on *S. aureus* was ambiguous. The essential oil also presented the activity to inhibit collagenase and α-glucosidase enzymes while the tyrosinase inhibitor was not detected. The collagenase inhibitory activity was IC_{50} 0.059 mg/mL and α-glucosidase inhibition was two-fold higher than the ability of acarbose, with an IC_{50} 0.677 mg/mL, respectively.

The antimicrobial activity was attributable to *trans*-anethole, consistent with prior reports of *Candida* species inhibition by anise-derived anethole [30]. Anethole has been reported to induce oxidative stress-dependent apoptosis in an opportunistic pathogenic fungus via mitochondrial death cascades [31]. The lipophilic and hydrophobic structures of the essential oil's chemical components directly interact with the fungal membrane, altering its properties and fluidity [32].

The collagenase inhibitory activity of *C. harmandiana* essential oil is an indicator of its potential future utility. Its ability to inhibit α-glucosidase was consistent with studies of other *Clausena* species (*C. indica* and *C. excavata*) that inhibit α-amylase and α-glucosidase [15, 33]. As well known, the mechanism of bioactive compounds that inhibit glucosidase can slow down the digestion and absorption processes of carbohydrates which is beneficial to decrease the blood sugar level. Interestingly, our result exhibited α-glucosidase inhibition two times higher than acarbose. Therefore, consumption of this plant as an edible or as a tea might indirectly help to control blood sugar level. Former study reported that 411 natural products isolated from medicinal plants showed anti-α-glucosidase activity. These compounds contained flavonoid, terpene, and phenylpropanoid ring structures [34]. This study will provide valuable information for further research, leading to the application of *C. harmandiana* as an alternative product development to control the level of blood sugar.

Table 3. The antimicrobial and enzymatic inhibitory activities of *C. harmandiana* essential oil.

Sample	Antimicrobial activity (mg/mL)				Enzymatic inhibitor (IC_{50} mg/mL)		
	S. aureus		*C. albicans*				
	MIC	MBC	MIC	MFC	Tyrosinase	Collagenase	α-Glucosidase
ECH	*	*	6.63	13.25	ND	0.059 ± 0.70	0.677 ± 9.21
Gen	0.02	0.02	-	-	-	-	-
Keto	-	-	0.04	0.08	-	-	-
Koj	-	-	-	-	0.015 ± 1.66	-	-
EGCG	-	-	-	-	-	0.005 ± 0.20	-
Acar	-	-	-	-	-	-	1.26 ± 4.30

*Ambiguous inhibition, ND: not detected, ECH: essential oil, Gen: gentamycin, Keto: ketoconazole, Koj: kojic acid, EGCG: epigallocatechin gallate, Acar: acarbose, -: not tested.

4. Conclusion

Fresh *C. harmandiana* leaves serve as a great source of the essential oil rich in *trans*-anethole. It possesses antioxidant, antimicrobial, and enzymatic inhibitory activities. These results provide information to support the utilization of this plant and future studies for the development of cosmetic and health care products, as well as medicinal applications of *C. harmandiana*.

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