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Antimalarial Evaluation of Cuminaldehyde, an Aromatic Monoterpenoid, using Cell Free β-hematin Formation Assay

Mahdi Mojarrab[a,b], Marieh Rezaei[c], Sayyed-Shahram Miraghaee[b], Gisya Abdi[d], Yalda Shokoohinia[a,b*]

[a] Novel Drug Delivery Research Center, School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran
[b] Department of Pharmacognosy and Biotechnology, School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran
[c] Student Research Committee, School of Pharmacy, Kermanshah University of Medical Sciences, Kermanshah, Iran
[d] Faculty of Chemistry, Department of Organic Chemistry, Razi University, Kermanshah 67149, Iran

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Malaria as one of the most recurrent infectious diseases caused by parasites of the genus *plasmodium,* kills several hundred thousand people especially in the tropical and subtropical regions of the world annually. Terpenoids have served as the lead compounds to develop new antimalarial agents. The aromatic monoterpenoid, cuminaldehyde, isolated from the fruits of *Bunium persicum* was evaluated for antimalarial activity using cell-free β-hematin formation assay. The purified compound showed no inhibitory performance with respect to β-hematin formation. It is presumably due to structural differences between cuminaldehyde and other known active terpenoids.

[*]**Corresponding Author: Yalda Shokoohinia**, **E-mail:** yshokoohinia@kums.ac.ir

Introduction

Malaria is an infectious disease of humans and other animals caused by parasitic protozoans of the genus *Plasmodium* [1]. The World Health Organization (WHO) has estimated that in 2013, about 3.3 billion people, half of the world's population, are at risk of malaria. In 2010, there were about 219 million malaria cases and an estimated 660 000 malaria deaths. People live in the poorest countries are the most vulnerable to malaria. In 2010, 90% of all malaria deaths occurred in the WHO African Region, mostly among children under five years old [2].

Since antimalarial drugs are unavailable or unaffordable to many who live in risky areas, the use of complementary medicines for malaria treatment is a useful option and *in vitro* screenings of plants serves as a key component of a critical path for antimalarial drug discovery [1,3]. Traditional medicines have been used to treat malaria for thousands of years and are the source of two main groups (artemisinin and quinine derivatives) of modern antimalarial drugs [4].

Plant volatiles usually occur as a complex mixture of low-molecular weight lipophilic compounds derived from different biosynthetic pathways, and are produced as part of a defense strategy against other microorganisms and environmental stress, as well as contributing to various physiological functions [5].Some essential oil components have shown anti-malarial effects [6, 7].

Terpenoids could be antimalarial lead compounds. Among them, triterpenoids [8], diterpenoids [9], sesquiterpenes (caryophyllene, α-farnesene, farnesol) [10], sesquiterpene lactones (artemisinin), monoterpenoids [10-12] could be mentioned.

Cuminaldehyde (Fig. 1) is an aromatic monoterpenoid mostly found in plants like *Cuminumcyminum* [13], *Carum carvi* [14], and *Cinnamomum cassia* [15], and has shown anti-platelet [16], antibacterial [13] andantifungal[17]effects. Besides, cuminaldehyde was useful in ameliorating symptoms of diabetes [18] and Parkinson's [19] (Table 1).

Bunium persicum fruits are used as cuminaldehyde source. Thefruit are used in bread, rice, cheese and yogurt processing for its carminative, antiflatulent, antispasmodic and antimicrobial effects [20], besides thepleasant smelling characteristic. Fruits essential oil is usually used for losing weight and as lactogogueas well [21].

B. persicum essessential oil has shown antioxidant [22-24] effects. Several analyses have been performed on essential oil of wildly growing or cultivated types of *B. persicum* which showed different chemical patterns, however, γ-terpinene and cuminaldehyde are among the most prevalent chemicals in the essential oil of this plant [25].

Hence, it seemed interesting to investigate the antimalarial potential of the monoterpenoid cuminaldehyde isolated from its source *Bunium persicum*. Different types of monoterpenoids with alkaloid [1] and glycoside [26] moieties, halogenated derivatives [27] and phenylpropanoid coupled monoterpenoids [28] have shown antimalarial effects, but this is the first report of antimalarial effect of an aromatic monoterpenoid.

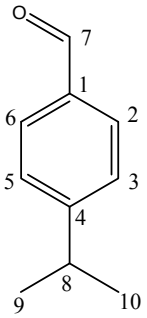

Fig. 1. Structure of cuminaldehyde

Materials and methods

General instruments

^{1}H-NMR (500 MHz) spectra were measured on a Bruker spectrometer. Chemical shifts were referenced to the residual solvent signal (CDCl3: δH 7.26) and TMS was used as an internal standard. EI-MS spectra were recorded on an Agilent 5975C mass spectrometer. Infra-red analysis was performed on IRPrestige-21, Shimadzu. Open column liquid chromatography was performed using silica gel (0.04-0.063 μm) or RP18 (0.015-0.030 μm); separations were monitored by TLC (Merck 60 GF_{254},0.25 mm) plates and were visualized by UV inspection and/or staining with Cerium sulfate/Molibdate and heating. The latter was prepared by adding 21 g Sodium Molibdate and 1 g Cerium Sulphate to 31 cc H2SO4 96%, and adding H2O to have 500 cc reagent mixture.

Table 1. Cuminaldehyde pharmacological review

Pharmacologic activity	Effect	Reference
Parkinson's disease management	Inhibition the fibrillation of alpha-synuclein, (major component of protein plaques in synucleinopathies particularly Parkinson's disease)	[19]
Antifungal	*Aspergilus niger*	[13]
Antibacterial	*Bacillus subtilis, Staphylococu sepidermidis* inhibition	[13]
Anti-yeast	*Saccharomyces cervisiae, Candida albicans* inhibition	[13]
Antibacterial	Food-borne bacteria inhibition	[34]
Antidiabetic	Aldose reductase inhibitor alfaglucosidase inhibitor	[18]

Plant Material

Fruits of *Bunium persicum* were collected from Mount Siahkooh, Sirjan, Iran, in May 2011 at an altitude of 1710 m above sea level. The plant identity was confirmed by the Botany Department of Isfahan University, Isfahan, Iran, and compared to voucher specimen in herbarium of Isfahan School of Pharmacy (No. 1712).

Extraction and isolation of cuminaldehyde

Dried ground fruits (1000 g) were extracted with acetone for two days (10L× 3). Then, it was concentrated to a viscous mass (60 g).Acetone extract was fractionated by VLC (RP18) using a gradient of MeOH: H2O from 3:7 to 10:0 to afford ten fractions (F1-F10).Fraction F8 was purified by using a gradient of Heptane: EtOAc(8:2 to 4:6) to obtain pure cuminaldehyde (100 mg). Purification of fraction F9 after normal phase chromatographies on Silica gel (Heptane: EtOAc, then toluene: Dichloromethane) resulted in more cuminaldehyde (1.5 g), overall equal to 0.16% w/w dry weight (Fig. 2).

Cuminaldehyde; cumin-7-al; 4-isopropyl benzaldehyde; clear colorless to yellow liquid.^{1}H-NMR: ($CDCl_3$, 500MHz): Table 2. IR (liquid film): 1740 cm^{-1} (CH=O), 1475, 1600 cm^{-1} (aromatic C=C), 900 cm^{-1} (aromatic OOP); EI-Mass (*m/z*): 148 $(M)^+$, 147 $(M-H)^+$, 105 $(M-C3H7)^+$, 91 (tropyllium)$^+$, 77 $(C6H5)^+$, 29 $(CHO)^+$.

Heme biocrystallisation and inhibition assay for potential antimalarial activity

The potential antimalarial activity of cuminaldehyde was evaluated by the method described by Fitch et al. [29] with some modifications [30]. Briefly, varying concentrations (0-2 mg/mL in 10% DMSO) of cuminaldehyde were incubated with 300 µM of hematin (freshly dissolved in 0.1 M NaOH), 10 mM oleic acid and 10 µM HCl. The reaction volume was adjusted to 1000 µL using 500 mM sodium acetate buffer, pH 5. Chloroquine diphosphate was used as a positive control. The samples were incubated overnight at 37 °C with regular shaking. After incubation, samples were centrifuged (14,000 x g, 10 min, at 21 °C) and the hemozoin pellet repeatedly was washed with sonication (30 min, at 21 °C in 2.5% (w/v) SDS in phosphate buffered saline followed by a final wash in 0.1 M sodium bicarbonate, pH 9.0, until the supernatant was clear (usually 3-5 washes). After the final wash, the supernatant was removed and the pellets were re-suspended in 1 mL of 0.1 M NaOH before determining thehemozoin content by measuring the absorbance at 400 nm using a 1 cm quartz cuvette. The results were recorded as % inhibition (I %) of heme polymerization/crystallization compared to positive control (chloroquine) using the following formula: I% = ((AB–AA)/AB) x 100, where AB: absorbance of negative control; AA: absorbance of test sample.

Table 2.^{1}HNMR data of cuminaldehyde, $CDCl_3$, 500 MHz.

H No.	δ (ppm)	Integral	Multiplicity, *J* in Hz
2	7.8	1	d, *J* = 8.15
3	7.4	1	d, *J* = 8.25
5	7.4	1	d, *J* = 8.25
6	7.8	1	d, *J* = 8.15
7	10.0	1	s
8	3.1	1	m
9	1.3	3	d, *J* = 6.93
10	1.3	3	d, *J* = 6.93

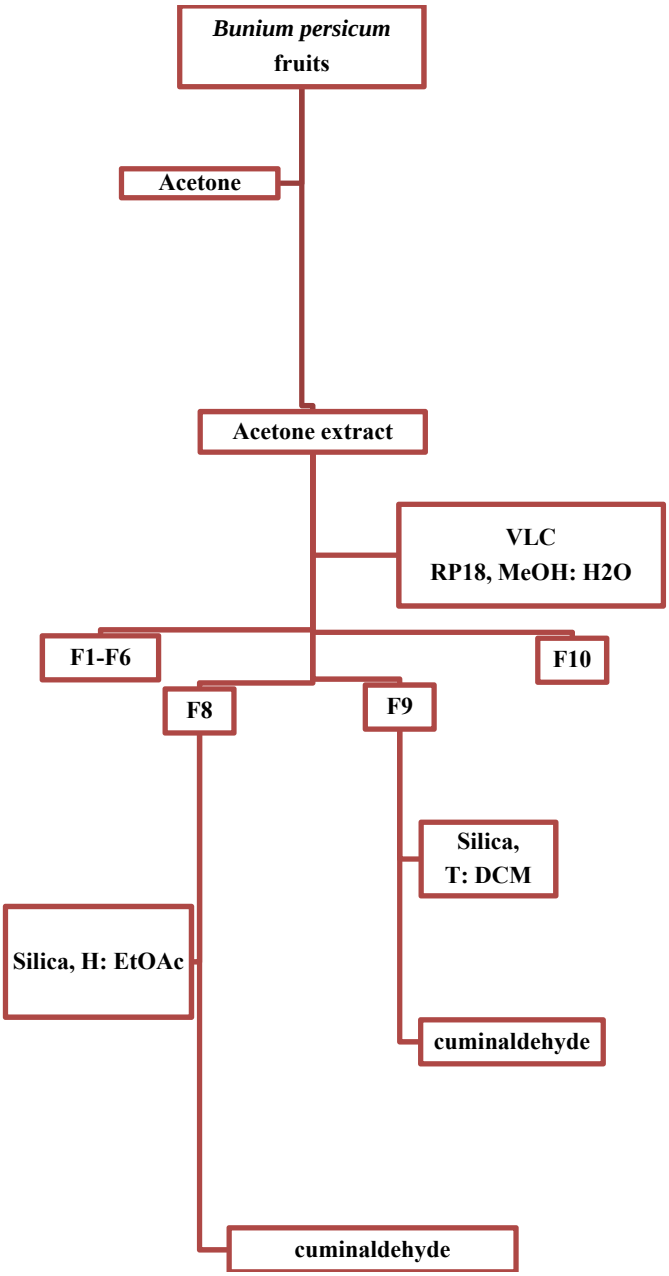

Fig. 2. Schematic flowchart of cuminaldehyde purification from *Bunium persicum* fruits; VLC: vacuum liquid chromatography; H: heptane; EtOAc: ethyl acetate; T: toluene; DCM: dichloromethane.

Results and discussion

Using acetone as extractor solvent and mixtures of MeOH: H2O on RP18 as primary chromatography solvent system, a mixture of nonpolar constituents was achieved, in which, the most nonpolar compound of some of the fractions after normal phase chromatographies determined to be cuminaldehyde. Finally, cuminaldehyde structure was confirmed by spectroscopic analyses. ^{1}HNMR analysis (Table 2, Fig. 1) showed some recognizable signals such as a singlet at 10 ppm for aldehyde proton (confirmed by IR and mass spectra), doublets at 7-8 ppm for aromatic protons, multiplet at 3.1 ppm for benzyl proton, and doublets at 1.3 ppm for methyl protons of isopropyl group with vicinal ^{1}H-^{1}H coupling constant $_{3}J$ HH = 1.3 Hz.

Compared to the results of positive control, cuminaldehyde did not show any decrease in UV absorbance in Heme biocrystallisation assay. That is why it is impossible to calculate and report IC50 value.

Terpenoids and phenyl propanoids are regarded as the major constituents of the essential oils which are in charge of reported biological effects for these volatile extracts [10]. (E/Z)- Nerolidol [31, 32] and farnesol [31] are examples of sesquiterpenoids which have the major role in displaying antiplasmodial effects of the essential oils. Monoterepene glycosides [26], their phenylpropanoid conjugated derivatives [28], halogenated [27] and alkaloid monoterpenes [1] as well as simple monoterpenoids like linalool [31]have exhibited antiplasmodial effects. To the best of our knowledge, there is no strong structural similarity between cuminaldehyde and monoterpenoids with proven antiplasmodial activity. Specially, none of them rather than cuminal has an aromatic cycle, the structural feature which presumably prevent the active engagement of the molecule in the antiplasmodial activity.

Several mechanisms are proposed for antimalarial effects of terpenoids. For example, artemisinin shows antiplasmodial effect through induction oxidative stress to iron rich parasites by producing cytotoxic radicals [33]. In comparison to other monoterpenoids, cuminaldehydeis special because of its aromatic ring and makes it resemble to phenyl propanoids. However, antimalarial effects of phenyl propanoids have been reported, in which they were monoterpenoid glycosides coupled to phenyl propanoidmoiety [28]. Anyway, although there is the aromatic ring in cumin aldehyde, it lacks some other features like external double bond and there is difference in number of carbons.

Conflict of Interest

Authors certify that no actual or potential conflict of interest in relation to this article exists.

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