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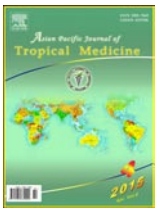

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Antiprotozoal assessment and phenolic acid profiling of five *Fumaria* (fumitory) species

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ABSTRACT

Objective: To explore some *Fumaria species* which were recorded to be traditionally used against malaria and other protozoal diseases. **Methods:** Consequently, in the current study, antiprotozoal effect of the ethanol extracts obtained from five *Fumaria* species (*Fumaria densiflora, Fumaria cilicica, Fumaria rostellata, Fumaria kralikii,* and *Fumaria parviflora*) was investigated against the parasites; *Plasmodium falciparum* (malaria) and *Trypanosoma bruceirhodesiense* (human African trypanosomiasis) at 0.81 and 4.85 μg/mL concentrations. **Results:** Among them, *Fumaria densiflora* extract exerted the highest antiplasmodial (93.80%) and antitrypanasomal effect (55.40%), while the ethanol extracts of *Fumaria kralikii* (43.45%) and *Fumaria rostellata* (41.65%) showed moderate activity against *Plasmodium falciparum*. Besides, phenolic acid contents of the extracts were analyzed using high performance liquid chromatography (HPLC) and *trans*-cinnamic (4.32 mg/g) and caffeic (3.71 mg/g) acids were found to be the dominant phenolic acids in *Fumaria densiflora*. **Conclusions:** According to our results, *Fumaria densiflora* deserve further study for its promising antiprotozoal activity.

1. Introduction

Fumaria species (Fumariaceae), known as "fumitory, earth smoke, beggary, fumus, vapor, fumittery or wax dolls", consist of a total of 46 species of annual herbs and common weeds in the world and many of them have been reported to have traditional utilization against hepato-biliary diseases throughout the world[1-4]. The genus *Fumaria*, named locally "sahtere" in Turkish, is represented by 16 species in the flora of Turkey[5] and several *Fumaria* species are consumed in tea form in Bosnia and Herzegovina, Turkey, and Cyprus[6-8], while the leaves of the plant are used in salads and eaten by French, Italians, Spanish, and Arabs in rural areas[9]. Various species of the plant have been reported to be used in folk medicine in India, Iran, and Pakistan against malaria and other parasitic diseases[3,10,11]. Besides, *Fumaria parviflora* (*F. parviflora*) was found to exert anti-nematocidal activity against in ruminants[12], while a notable antimycobacterial effect was observed with *Fumaria officinalis* (*F. officinalis*)[13].

Malaria caused by *Plasmodium falciparum* is a vectoral disease frequently seen in sub-Saharan African and Asian countries. According to WHO statistics, around 34.8 million cases and 45 600 deaths attributable to malaria were reported in the Asia region during 2010, where over 85% of the cases happened in India, Indonesia, Myanmar, and Pakistan[14,15]. On the other hand, sleeping sickness, also known as human African trypanosomiasis (HAT), is another neglected vectoral disease caused by *Trypanosomabrucei* which is common in tropical regions of the world, particularly prevalent in Africa continent[16]. For instance; it was documented that more than 1 000 new cases *per* year was declared only in the Central African Republic and Congo in 2009[17]. Malaria and trypanosomiasis, therefore, still constitute a major health problem since the drugs used against these parasites seems to be often unsuccessful and possess high toxicity. Furthermore, another problem with the current antimalarial and antitrypanosomal drugs is occurrence of

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quick resistance, which increases demand to development of more effective, less toxic, and inexpensive drugs.

Taking the traditional use of *Fumaria* species against malaria and other parasitic diseases into consideration, our goal was to test the ethanol extracts of five *Fumaria* species [*Fumaria cilicica* Hausskn. (FC), *Fumaria densiflora* DC. (syn. *Fumaria micrantha* Lag.) (FD), *Fumaria kralikii* Jordan (syn. *Fumaria anatolica* Boiss.) (FK), *Fumaria parviflora* Lam (FP), and *Fumaria rostellata* L (FR)] against the malaria vector; *Plasmodium falciparum* as well as *Trypanosoma brucei rhodesiense* (*T. brucei rhodesiense*); the factor of human African trypanosomiasis and identify their phenolic acid profile using HPLC.

2. Material and methods

2.1. Plant materials

The aerial parts of the *Fumaria* species were collected from various locations throughout Turkey and identified by Prof. Dr. Bilge Sener of Department of Pharmacognosy, Faculty of Pharmacy, Gazi University (Ankara, Turkey). Voucher specimensare preserved at the Herbarium of Faculty of Pharmacy of Gazi University (Ankara, Turkey).

2.2. Preparation of extracts

The plant materials were dried in the shade; powdered, and weighed accurately in a digital balance. Then, the plant samples were soaked in ethanol (85%) for 3 days and shaken by hand occasionally. Following filtration, the ethanol phase of each species was evaporated *in vacuo* until dryness and the crude ethanol extracts were obtained. The extract yields (w/w) were calculated as follows; FC: 30.86%, FD: 33.12%, FK: 35.97%, FP: 30.08%, and FR: 32.29%.

2.3. Antitrypanosomal activity test

Minimum essential medium supplemented according to Baltz *et al*[18] with 2-mercaptoethanol and 15% heat-activated horse serum was added to each well of a 96-well microtiter plate. Serial drug dilutions were prepared within a range between 90 μg/mL and 0.123 μg/mL. Afterward, 10^4 bloodstream forms of *T. brucei rhodesiense* STIB 900 were added into each well and the plate incubated at 37 ℃ under a 5% CO_2 atmosphere for 72 hours. Alamar Blue dye was then added to each well again and incubation was applied for another 2-4 hours. Following this duration, the plate was read in a ELISA microplate reader (SpectraMax Gemini XS microplatefluorometer, Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wavelength of 536 nm and emission wavelength of 588 nm[19]. Fluorescence development was expressed as percentage of the control.

2.4. Antimalarial activity test

Antimalarial activity was tested against the K1 strain of *Plasmodium falciparum* (resistant to chloroquine and pyrimethamine) by a modified (^{3}H)-hypoxanthine incorporation method[20]. To sum up, infected human red blood cells in RPMI 1640 medium with 5% Albumax were exposed to serial drug dilutions in microplates. Following 48 hours of incubation at 37 ℃ in a reduced oxygen atmosphere, 0.5 μCi ^{3}H-hypoxanthine was put into each well. Cultures were incubated for another 24 hs before they were harvested. The radioactivity was counted using a Betaplate™ liquid scintillation counter (Wallac, Zurich, Switzerland). The results were calculated as counts *per* minute (CPM) per well at each concentration and expressed as percentage of the untreated controls.

2.5. HPLC analysis conditions for phenolic acids in the extracts

An HPLC system consisting of a quaterner G1311A HPLC pump, model 1100 Autosampler, model G 1315B photodiode array detector was used for the analysis [all from Agilent GL Sciences Inc (Waldbronn, Germany)]. Standards and the *Fumaria* extracts were analyzed on a reverse-phase Zorbax Eclipse XDB-C18 column (150 mm, 4.6 mm *id*. and particle size 3 μm) (Agilent, Waldbronn, Germany). Ultra-pure deionized water was purified by Synergy Water Purification System (Millipore, Rotterdam, Netherlands) to a specific resistance of 18 mO cm. Chromatographic analysis of the extracts was carried out by a gradient elution [solution A, methanol:water:formic acid (10:88:2 by volume); solution B, methanol: water: formic acid (90:8:2 by volume)] as reported in our previous publication[21]. The flow-rate was 1 mL/minute, and the injection volume was 10 μL. Signals were detected at 280 nm. The internal standard technique was applied to increase the repeatability. The relevant extracts were dissolved in a mixture of methanol and

Table 1
Antiprotozoal activity (%) of *Fumaria* extracts.

Extracts	*Plasmodium falciparum* (Malaria)		*T. brucei rhodesiense* (Human African trypanosomiasis)	
	0.81 μg/mL	4.85 μg/mL	0.81 μg/mL	4.85 μg/mL
FC	0.00	29.85	7.15	10.70
FD	27.70	93.80	12.65	55.40
FK	21.30	43.45	13.40	11.60
FP	0.00	18.70	5.60	11.25
FR	11.75	41.65	7.65	12.70
Melarsoprol[a]			-[b]	99.50
Artemisinin[c]	-	99.70		

[a]Reference against *T. brucei rhodesience*; [b]Not determined.

Table 2
Phenolic acid quantities in *Fumaria* extracts analyzed by HPLC.

Extracts	GA	proCA	CA	*p*-COU	FA	*o*-COU	*tr*-CIN
FC	0.97	1.30	6.53	12.44	1.89	14.23	2.78
FD	0.45	0.81	3.71	1.31	1.50	2.01	4.32
FK	-	0.86	4.54	5.44	1.23	6.35	-
FP	-	0.64	5.18	2.16	1.13	2.12	1.30
FR	0.69	0.96	4.87	8.80	1.38	8.48	1.09

*The phenolic acid standards used aregallic acid (GA), protocathechuic acid (proCA), caffeic acid (CA), *p*-coumaric acid (-COU), ferulic acid (FA),o-coumaric acid (*o*-COU), and *trans*-cinnamic acid (*tr*-CIN).

water (1:1 vol/vol), and the mixture was injected into the HPLC apparatus (Figure 1).

3. Results

Among the ethanol extracts of five *Fumaria* species tested, FD extract showed the highest antiprotozoal effect (93.80%) at concentration of 4.85 μg/mL, followed by FK (43.45%) and FR (41.65%) (Table 1). Occurrence of the highest antitrypanasomal activity was also observed with the ethanol extract of FD (55.40%). Our HPLC analysis indicated that the richest extract considering phenolic acid quantity belonged to FC and FR (Table 2, Figure 1).

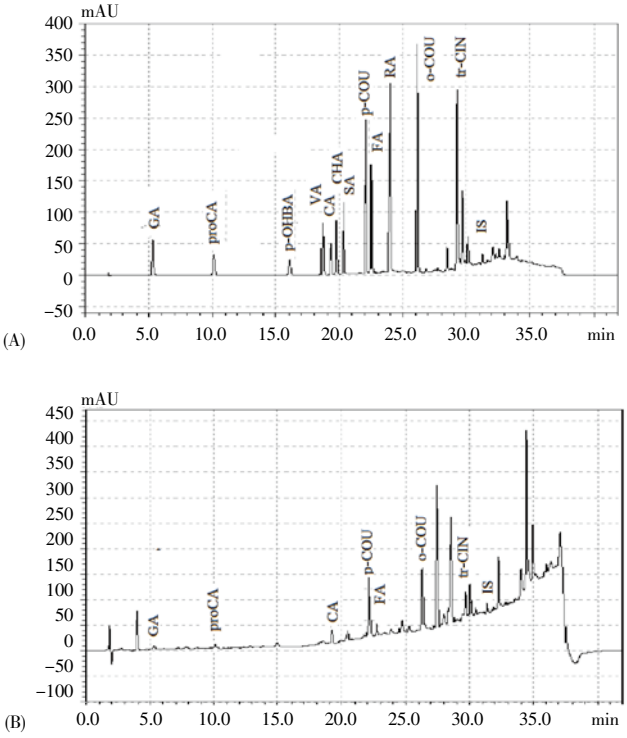

Figure 1. HPLC chromatogram of the reference phenolic acids used (A) and representative HPLC chromatogram of the ethanol extract of FC (B).
Abbreviations of the phenolic acid references used are gallic acid (GA), protocathechuic acid (protoCA), *p*-hydroxybenzoic acid (*p*-hydBA), vanillic acid (VA), caffeic acid (CA), chlorogenic acid (ChA), syringic acid (SA), p-coumaric acid (-COU), *o*-coumaric acid (*o*-COU), *trans*-cinnamic acid (*tr*-CIN) and ferulic acid (FA).

4. Discussion

Although some *Fumaria* species have been used in folk medicine against malaria in India, Iran, and Pakistan[3,10,11], we have not encountered any report about antimalarial and antitrypanasomal activities of *Fumaria* species up to date according to our literature survey. The genus *Fumaria* is well-known for its prosperous alkaloid content[22]. For instance; *Fumaria densiflora*, identified as the most active antimalarial extract herein, contains many isoquinolinealkaloids such as protopine, cryptopine, coptisine, palmatine, adlumidiceine, (+/-)-sinactine, fumafalorine, densiflorine, *etc*[23,24], while similar isoquinoline derivatives were also isolated from *Fumaria kralikii* and *Fumaria parviflora*[25]. In fact, several isoquinoline-type of alkaloids such as bidebiline E[26], 1-(4-hydroxybenzyl)-6,7-methylenedioxy-2-methylisoquinolinium trifluoroacetate[27], (+)-N-methylisococlaurine, atherosperminine, and 2-hydroxy-atherosperminine[28], and (-)-milonine were also reported from various other plant genera with antiprotozoal effect[29]. Therefore, it may be speculated that isoquinoline alkaloids seem to be the most responsible for the antimalarial effect of the *Fumaria* species.

According to our literature survey, only one report seems to be available on phenolic acid content of *Fumaria* species. In that early study by Sousek *et al*[30], presence of citric, coumaric, ferulic, fumaric, malic, S-hydroxybenzoic, protocatechuic, and caffeic acids were detected in several *Fumaria* species including FD and FP using gas chromatography-mass spectrometry (GC-MS), which is in accordance with our existent results. On the other hand, cinnamic acid derivatives have been stated to possess antiprotozoal activity presumably acting through inhibition of monocarboxylate transport and, therefore, blocking growth of intraerythrocytic *Plasmodium falciparum*[31]. *p*-Hydroxy-cinnamic acid also showed antiplasmodial effect against the multidrug-resistant W2mef strain of *Plasmodium falciparum*[32]. Since cinnamic acid is the most abundant phenolic acid in FD, the most active species in the current study, it might be contributing to the antiprotozoal effect of FD, at least in part. Consequently, the data obtained from this study confirms the antimalarial use of *Fumaria species* in folk medicine as its extracts displayed a notable profile in antimalarial assays.

In conclusion, the screening results suggest that the ethnopharmacological use of *Fumaria* species against malaria can be confirmed on a scientific base and the plant deserve further studies to be evaluated as promising antimalarial agent. To the best of our knowledge, this is the first study on antiprotozoal activity and phenolic acid profile of FC, FD, FK, FP, and FR.

Conflict of interest statement

We declare that we have no conflict of interest.

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