

Safflower (*Carthamus Tinctorius* L.) a Potential Source of Drugs against Cryptococcal Infections, Malaria and Leishmaniasis

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Received: 21 June 2019; Accepted: 18 September 2019

Abstract: In this research we present that *Carthamus Tinctorius* L. (gen. Asteraceae, otherwise known as Safflower) (Fig. 1) may contain agents active in Cryptococcal infections, malaria and Leishmaniasis, as treatment options are becoming scarce due to drug resistance development. Phytochemistry and pharmacological activities (antimicrobial, antimalarial, antileishmanial) of *C. tinctorius* L. were analyzed. The composition of volatile oil of safflower dried flowers was analyzed by gas chromatography-mass spectrophotometry with flame ionization detector (GC-FID) and in vitro sensitivity assays were performed to assess biological activity. 8 known and 3 unknown compounds were detected in the extract (Fig. 1). Then the Safflower ointment was manufactured and its acute toxicity study on rats was tested. The volatile oil of *C. tinctorius* L. exhibited activity against *Cryptococcus neoformans*, *Plasmodium falciparum* and *Leishmania donovani*. Safflower volatile oil has anticryptococcal, antimalarial and antileishmanial effects. The prepared ointment had an excellent acute toxicity safety profile.

Keywords: *Carthamus tinctorius* L.; Safflower; volatile oil; GC-FID; biological activity

1 Introduction

C. tinctorius L. (gen. Asteraceae, otherwise known as Safflower) (Fig. 1), is mainly grown for its seed, which is edible, or its flowers, that may be used for colouring, flavouring foods or medicinal purposes [1]. The standard safflower oil contains about 6-8% palmitic acid, 2-3% stearic acid, 16-20% oleic acid and 71-75% linoleic acid. The Safflower has also been used in folk medicine as an analgesic, antithrombotic and antihypertensive remedy [2-4]. In some regions of Africa and Asia, the Safflower has been used as an antidote to poison and laxatives, also it is used as a sweetener or an antipyretic [5]. Many compounds have been isolated from *C. tinctorius* L. including flavonoids, phenylethanoid glycosides,



coumarins, fatty acids, steroids and polysaccharides [6-8]. It was also found, that *C. tinctorius L.* increases hair growth in mice, while the hydroxysafflor yellow A, found in Safflower, affects the mRNR expression of keratinocyte and vascular endothelial growth factors [5].

More than 200 various compounds, isolated from *C. tinctorius L.*, including the fatty acids, steroids, flavonoids, coumarins, polysaccharides, have antimicrobial properties against *Bacillus subtilis*, *Bacillus cereus*, and *Bacillus mycoides*, as well as some fungi, especially *Aspergillus niger*, *Penicillium expansum*, *Geotrichum candidum*, *Aspergillus fumigatus*, *Candida albicans*, and *Rhodotorula rubra* [9-12]. Recently it has been shown that this plant has analgesic and anti-inflammatory activity due to safflomin A and safflomin B [7], antidiabetic activities and may aid in the treatment of osteoporosis. The safflower seed oil has beneficial effects in osteoporosis, due to high linoleic acid levels. Moreover, the *C. tinctorius L.* flowers has an anticoagulant effect and is used to promote the blood circulation [9-12]. In addition, the *C. tinctorius L.* contains phenolic and flavonoid substances and exhibits radical scavenging and oxygen radical absorbant properties and because safflower extract reduces oxidative stress it is used to prevent and treat some cardiac diseases [7,8,13]. The phytochemical and biological properties of the essential oil of *C. tinctorius L.*, grown in Kazakhstan, may be different than that, reported in previous research due to the geographic and climatic factors, chemo types, drying conditions and mode of distillation as this commonly occurs with other herbs [14-17].

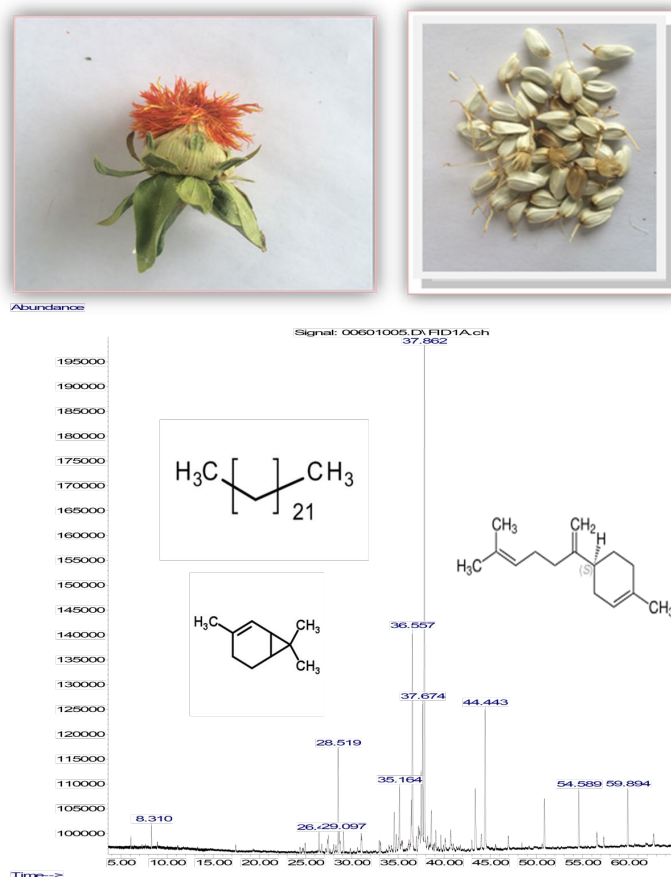


Figure 1: *Carthamus tinctorius L.* and its chemical composition

The potential clinical relevance. The Cryptococcal infections occur in patients with immunosuppression: in the HIV infected patients or in cases of solid organ transplantation [18]. Some studies showed that safflower can increase survival in patients, that have severe sepsis and septic shock. In patients with sepsis or septic shock, the safflower improves respiratory and cardiovascular functions

and decreases inflammation [19]. The *Cryptococcus sp.* are inherently resistant to echinocandins and readily develop resistance to fluconazole; such condition is difficult to manage [20]. On the other hand, less toxic drugs than amphotericin B are needed for the management of cryptococcosis [21]. Malaria resulted in 429,000 deaths in 2015 [22]. Resistance to the antimalarial drugs (chloroquine and sulfadoxine-pyrimethamine) is also currently on the rise, thus research to identify new antimalarial compounds is well justified [23,24]. Leishmaniasis is a tropical disease caused by the protozoa *Leishmania* and is transmitted by infected sandflies [25]. Antimonials (sodium stibogluconate) are the primary drugs employed against leishmaniasis and resistance to them is also currently developing [26]. Thus, we evaluated the anti-cryptococcal, antimalarial and antimonial properties of the Safflower.

2 Materials and Methods

2.1 Plant Material

The plant material (Safflower flower) was collected from the Southern Kazakhstan (Almaty) region during the flowering stage. The plant was identified by the taxonomist Dr. Konyrbekov M. A voucher specimen was deposited at the herbarium Krasnovodopadskaya Breeding Experimental Station, Ministry of Agriculture, Republic of Kazakhstan. Dry plant material (seeds and petals) were collected in the summer of 2015, and were subject to treatment and disposal of solids, drying, then grinding before the experiments. The voucher specimen number: State standard of the Republic of Kazakhstan. Seeds of oil various cultures. Seeds of Safflower CT PK 1364-2005 (ST RK 1364-2005), Patent No. 28798. (https://online.zakon.kz/Document/?doc_id=30088581#pos=0;20).

2.2 Extraction of Essential Oils

The dried flower was subjected to hydrodistillation in a Clevenger type apparatus for 4 hours. At the end of the distillation, the oil was collected, dried with anhydrous Na_2SO_4 , transferred to glass vials and kept at temperature of -18 degree Celsius for further analysis.

2.3 Gas Chromatography-Mass Spectrophotometry with Flame Ionization Detector (GC-FID)

The oil samples were analyzed with the GC-FID on the Agilent 5975 C inert XL MSD with the triple axis detector/7890A GC system equipped with the Agilent 7693 Autosampler, a DB-WAX column (30 m \times 0.32 mm, 0.5 mm thickness) [27], operated using the following conditions: injector temperature, 240°C; column temperature, 40-120 at 3°C/min, then held at 240°C at 20°C/min for 5 min; carrier gas, He; injection volume, 1 μl (split on FID, split ratio 50:1); FID temperature was 300°C [28]. The compounds were identified in oil samples by Kovat analysis and the comparison of mass spectra of the identified compounds with those reported in the NIST mass spectra database. The compounds were quantified by performing the area percentage calculations, based on the total combined FID area [14].

2.4 Identification of Components

The retention times, Kovats indices and mass spectra were used to identify the components of the oil. The confirmed integrated peaks were used to evaluate the percentage of each chemical component found in the Safflower essential oil. Kovats indices were estimated using the equation: $KI(x) = 100 [(\log RT(x) - \log Pz) / (\log RT(Pz + 1) - \log RT(Pz))]$, where: $RT(Pz) \leq RT(x) \leq RT(Pz + 1)$, and $P4, \dots, P25$ are n paraffins [14].

2.5 Preparation of the Ointment

The optimal ointment in terms of the physical, chemical, technological, structural, mechanical, and microbiological properties was made and consisted of (per 100.0 grams of ointment): the active substance essential oil obtained from the flowers of safflower 9.0 g and the auxiliary substances: sunflower oil 40.0 g, emulsifier T-2 5.0 g, Purified Water 46.8 g, Oleum Menthae piperitae 0.2 g.

2.6 Ethical Statement

The animal experiments were carried out in accordance with the Guide for the Care and the Use of Laboratory Animals, published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996), the ARRIVE guidelines and were within the rules of humane treatment of animals, regulated by the Federal Law “Animals protection against cruel treatment” from 01.01.1997 and the provision of the European Convention for the Protection of Vertebrate Animals. The acute toxicity study of the ointment was performed in accordance with the procedure description in the “Comprehensive Guide to Toxicology in Nonclinical Drug Development (Second Edition)” [29].

All the experiments were approved by the Local Ethics Committee of Asfendiyarov Kazakh National Medical University (Protocol #1, 29.01.2015).

2.7 Acute Toxicity Study

The Acute toxicity study of the ointment (described in 2.5) was tested on the experimental animals to evaluate the local tolerance of the ointment. The ointment was applied locally to the outer cover of the tail of rats. In the experiment, 24 male and female Wistar rats (weighing 200-260 g) were included: group 1 received 1.24 g of the ointment (locally) and the group 2 received 2.72 g of the test ointment (locally). The animals were monitored for 14 days for the development of local (redness or swelling) or other systemic signs of irritation.

2.8 Pharmacological Actions

2.8.1 Antimicrobial Assay

The Safflower volatile oil was tested for antibacterial activity with broth dilution test [30] against the gram positive staphylococci (*S. aureus* ATCC 29213 and methicillin resistant *S. aureus* ATCC 33591(MRS)), the gram-negative bacteria (*Escherichia coli* ATCC 35218 and *Pseudomonas aeruginosa* ATCC27853), the acid-fast bacilli (*Mycobacterium intracellulare* ATCC 23068). The antifungal activity was tested against the *Candida albicans* ATCC 90028, *C. glabrata* ATCC 90030, *C. krusei* ATCC 6258, *Cryptococcus neoformans* ATCC 90113, *Aspergillus fumigatus* ATCC 204305. The ciprofloxacin and amphotericin-B were used as positive controls for bacteria and fungi, respectively [31].

2.8.2 Antimalarial Activity

In vitro antimalarial activity was determined with broth dilution test [30] against the *P. falciparum* strains that are chloroquine sensitive (D6, Sierra Leone) and resistant (W2, Indo China) by measuring plasmodial LDH activity. Chloroquine was used as positive control [31].

2.8.3 Antileishmanial Activity

The antileishmanial activity was tested with broth dilution test [30] against *Leishmania donovani* promastigotes; pentamidine and Amphotericin-B were used as positive controls [31].

2.8.4 Statistical Analysis

The data was analyzed using the SPSS Statistics package (version 17) to calculate the descriptive statistics.

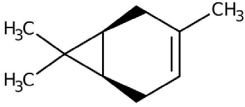
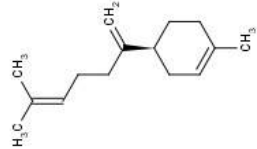
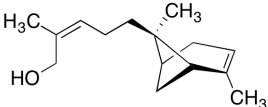
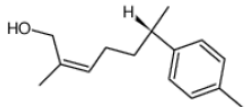
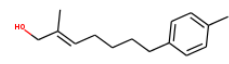
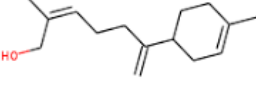


3 Results

3.1 Extraction Yield and GC-FID Analysis of Volatile Oil Components

The *C. tinctorius* L. flower oil was analyzed by GC/MS. A total of 8 components were identified (3-carene, beta-bisabolene, alpha-trans-bergamotol, Z-nuciferol, E-nuciferol, cis-Lanceol, n-tricosane and pentacosane) and three unknown compounds were quantified in safflower essential oil. The 8 components

represented about 85.283% of the total detected components (Tab. 1, Fig. 2).

Table 1: Chemical composition of *C. tinctorius L.* essential oil

Compounds	Description	Chemical structure	Retention time	Kovat Index (KI)	Area %
3-Carene	Is a bicyclic monoterpene and contains 2 rings, which are dissolved with each other. Carene has a sweet and pungent odor and is a flavouring ingredient. It is mixable with oils or fats, but is not soluble in water [32].		8.310	1007.70	0.967
Unknown	-		26.458	1468.93	1.358
Beta-Bisabolene	A common monocyclic sesquiterpene. Beta-Bisabolene is a plant metabolite which is produced during metabolic reaction in plants [33].		28.519	1512.75	6.629
Unknown	-		29.097	1526.91	1.392
Alpha-trans-bergamotol	It is bicyclic monoterpene. It can be found in cereals and their products. Alpha-Bergamotenol is also a flavouring ingredient [34].		35.164	1686.46	3.457
Z-Nuciferol	It can also be found in sandalwood (<i>Santalum spicatum</i>) essential oil [35].		36.557	1725.99	14.139
E-Nuciferol	It is sesquiterpenoid and can be found in <i>M. micrantha</i> essential oil [36].		37.674	1755.92	9.057
Cis-Lanceol	It can be also be found in sandalwood (<i>Santalum spicatum</i>) essential oil [37].		37.862	1761.72	42.449
Unknown	-		44.443	1957.40	11.967
n-Tricosane	Belongs to the class of acyclic alkanes. And is a straight chain alkane which contains 23 carbon atoms. N-Tricosane is a plant metabolite and a volatile oil component [38].		54.589	2299.22	4.197
Pentacosane	It is a straight chain alkane which contains 25 carbon atoms. Pentacosane has a role as a plant metabolite as well as a semiochemical [39].		59.894		4.388

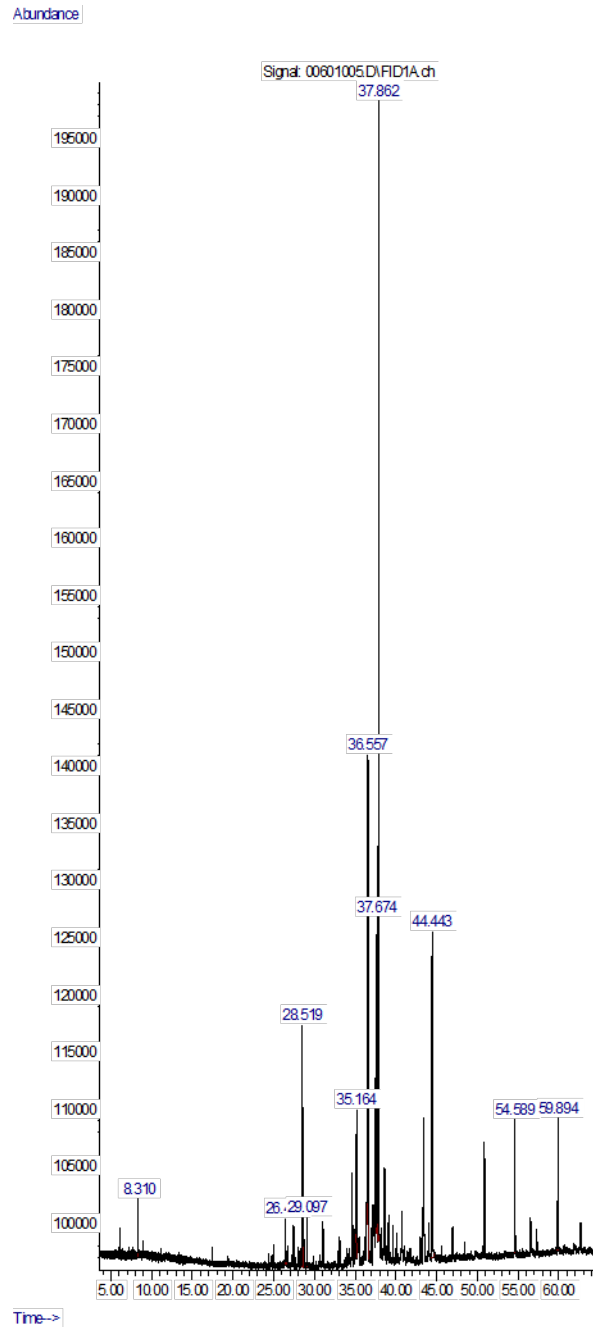


Figure 2: The GC FID of *C. tinctorius L.*, essential oil

3.2 Antimicrobial Properties

The oil exhibited good activity against *Cryptococcus neoformans* ATCC 90113 with an IC₅₀ value of 8 µg/ml. However, no other antimicrobial activity of *C. tinctorius L.* was identified (Tab. 2).

Table 2: Biological activity of essential oil of *Carthamus tinctorius L.*

Biological activity	Test parasite	<i>Carthamus tinctorius L</i> essential oil ($\mu\text{g/ml}$)
Antileishmanial activity	<i>L. donovani</i> IC ₅₀	80
Antimalarial activity	<i>P.falciparum</i> D6 IC ₅₀ W2 IC ₅₀	47600
Antimicrobial activity	<i>C .neoformans</i> IC ₅₀	20.41

3.3 Antimalarial Activity

The volatile oil of the *C. tinctorius L.* showed weak antimalarial activity (IC₅₀ 47600 $\mu\text{g/ml}$) against chloroquine sensitive *P. falciparum* (D6) and chloroquine resistant *P. falciparum* (W2) (Tab. 2).

3.4 Antileishmanial Activity

The oil of *C. tinctorius L.* was moderately effective against the *Leishmania donovani* promastigotes with IC₅₀ value of 80.0 $\mu\text{g/ml}$ (Tab. 2).

3.5 Acute Toxicity Study

During the acute toxicity study rats developed no systemic or local adverse events. Animals in both groups remained active, and there was no case of death or poisoning. The results of the experiment showed the absence of pathological changes in the nature of general and specific indicators over the entire study period. The animals in all groups remained active and there was no case of death or poisoning. The local administration of the prepared ointment may be considered as safe (within the limits of the preclinical nature of this study).

4 Discussion

Compound extraction. The 8 known and 3 unknown compounds were detected in the *C. tinctorius L.* extract. The major compounds of the oil were 3-carene (mainly used as a flavouring ingredient), beta-bisabolene (mainly used as a flavouring agent), alpha-trans-bergamotol (a sesquiterpenol [40], as such may exhibit anti-inflammatory properties [41]), Z-nuciferol (an essential oil), E-nuciferol (an essential oil), cis-Lanceol (an essential oil), n-tricosane (an acyclic alkane) and pentacosane (constituent of many naturally occurring waxes) [42]. The specific descriptions of the pharmacological effects of these compounds are rather scarce in the scientific literature.

The antimicrobial study of the *C. tinctorius L.* volatile oil revealed the potent activity against the *cryptococcus neoformans* and absence of antimicrobial activity. Previous research failed to uncover the activity against dermatophytes (*trichophyton mentagrophytes*, *microsporum canis* etc.), filamentous fungi (*aspergillous niger*, *A. fumigatus*) and yeast (*Saccharomyces cerevisiae*, *C. neoformans*, *Candida albicans*) [2,43]. Although some studies showed that safflower has antimycotic properties, especially against the *Aspergillous fumigatus* [7].

We also show, that the volatile oil of *C. tinctorius L.* has a weak activity against both the chloroquine sensitive and resistant *Plasmodium falciparum*. Similar results are described by other authors [43-45]. The antileishmanial evaluation showed moderate activity of the oil *C. tinctorius L.* against *Leishmania donovani*. The in vitro inhibitory effect of *C. tinctorius L.* extracts against the *L. major* promastigotes has

also been previously demonstrated [46]. The ointment made of the Safflower flowers may have antimicrobial properties and is well tolerated upon local administration.

5 Conclusions

The results of the study show that the safflower, collected in the southern region of Kazakhstan, may have therapeutic benefits (arising from antimicrobial, antimalarial and antileishmanial effects) and is a valuable plant, that may serve a source of inspiration for the modern drug development. The identification of specific compounds in the extract helps to understand the pharmacologic basis of Safflower biological action. It seems that *C. tinctorius L.* grown in Southern region of Kazakhstan is likely to be non-inferior with respect to the phytochemical composition and pharmacological activity to *C. tinctorius L.* grown elsewhere.

Acknowledgement: The authors thank for the financial support offered by the Kazakhstan Government and National Center for Natural Product Research, University of Mississippi, Mississippi, USA.

Authors' Contributions: Turgumbayeva A. Ustenova G., Rakhimov Kh. D., Zhakipbekov K. S., Kalykova A.S., Ustenova O., Abramavičius S., Tunaitytė A., Stankevičius E. performed the experiments, statistical analysis and contributed to manuscript preparation. Samir A. was responsible for the technical support.

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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