

Determination of the chemical composition of the essential oil of the *Rubia tinctorum* plant

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From past to present, plants and plant extracts have been used in many scientific and commercial fields for various purposes. *Rubia tinctorum* L. (Rubiaceae) has a long history of traditional use as a diuretic and in the treatment of conditions such as jaundice, sciatica, and paralysis. In this study, the essential oil of *R. tinctorum* was obtained by the hydrodistillation method, and the volatile and semi-volatile compounds it contains were identified using the GC–MS technique. GC–MS analysis revealed the presence of 21 compounds in the essential oil extract of *R. tinctorum*. The chemical profile was determined by fatty acids, accounting for 89.29% of the total composition, followed by alkanes (5.32%), terpenoids (4.57%), alcohols (0.5%), and aldehydes.

Keywords: *Rubia tinctorum*, essential oil, gas chromatography, GC-MS.

INTRODUCTION

Globally, estimated 50.000–70.000 plant species are utilized for medicinal applications, while nearly 500 medicinal plants are used therapeutically in Türkiye [1]. Interest in plant-derived natural products has been steadily increasing in recent years. Plants have gained considerable scientific interest because of their wide range of biological properties, such as anti-inflammatory, anticarcinogenic, antifungal, antiallergic, antioxidant, and antibacterial activities.

Consequently, research focusing on the isolation of biologically active compounds from plants and the elucidation of their chemical structures has intensified in recent times.

The genus *Rubia* belongs to the Rubiaceae family, which comprises approximately 450 genera and around 6.500 species. The genus *Rubia* is widely distributed, encompassing over 60 species found in Africa, temperate regions of Asia, and the Americas. In India, about 15 species of *Rubia* have been reported [2]. Species of the genus *Rubia* (Figures 1 and 2) are of considerable commercial, economic, and medicinal importance, primarily due to their high anthraquinone content, which is predominantly accumulated in the roots [3].

Extracts obtained from *R. tinctorum* L. contain several bioactive constituents with notable pharmacological relevance. Traditionally, crude

extracts have been used to treat bladder and kidney stones, especially calcium oxalate and calcium phosphate calculi in the urinary tract. *In vitro* investigations have shown that ruberythric acid suppresses the formation of calcium oxalate and calcium phosphate crystals [5-8].



Figure 1. Aerial parts of *R. tinctorum* [4]



Figure 2. Roots of *R. tinctorum* [4]

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R. tinctorum (Rubiaceae) has traditionally been used as a diuretic and for the treatment of ailments including jaundice, sciatica, and paralysis. It has also been utilized to regulate menstruation after childbirth and to manage conditions such as rheumatism, eczema, and fungal infections [9]. Moreover, *R. tinctorum* serves as an important natural source of dye raw materials utilized in the cosmetic, textile, food, and pharmaceutical sectors. Beyond its industrial applications, the species is also well known for its diverse and beneficial biological activities [10].

The present study aims to determine the secondary metabolites found in the essential oil of *R. tinctorum* L. cultivated in the Edirne - Havsa region by using the GC–MS method. In this context, the study will contribute to the chemotaxonomic classification of *R. tinctorum*.

MATERIALS AND METHODS

Plant material and extractions

The cultivated *Rubia tinctorum* was harvested in August 2024 from the garden of Trakya University Havsa Vocational School and subsequently dried in a shaded, well-ventilated environment. The dried material was then divided into aerial parts and roots. After the aerial part of the plant was cut into small pieces, it was placed in a Clevenger apparatus and subjected to steam distillation for 12 h. Subsequently, extraction was performed on the water-oil mixture, yielding 0.074 g of crude essential oil [11].

Determination of volatile and semi-volatile compounds in essential oil of R. tinctorum by GC-MS

To determine the composition of the compounds present in the essential oil extract of the plant, a Shimadzu QP2010-Ultra model GC–MS instrument was used. GC–MS analyses were performed using a TRB-5MS capillary column (30 m × 0.25 mm × 0.25 µm) coated with 5% phenyl–95% methylpolysiloxane. The split ratio was adjusted to 10:1, with an injection volume of 1 µL. The injector temperature was kept at 250 °C, while both the ion source and interface temperatures were maintained at 280 °C. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The initial column temperature was set at 50 °C and held for 3 min. It was then increased at a rate of 3 °C/min to 100 °C and held for 5 min. Subsequently, the temperature was raised at 6 °C/min to 250 °C and held for 14.5 min. The mass spectrometer was operated in scan mode over a mass range of 45–700

m/z. The total analysis time was 64 min. The mass spectra of the compounds were identified by comparison with the Wiley 9 and NIST mass spectral libraries. In addition, the retention indices of each component in the mixture were calculated based on their retention index using a C6–C22 n-alkane standard mixture [12].

RESULTS AND DISCUSSION

GC–MS analysis revealed the presence of 21 compounds in the essential oil extract of *R. tinctorum*. The chemical profile was dominated by fatty acids, accounting for 89.29% of the total composition, followed by alkanes (5.32%), terpenoids (4.57%), alcohols (0.5%), and aldehydes (Table 1). This finding is consistent with previous reports on species within the *Rubia* genus, where fatty acids have been identified as major constituents of volatile and semi-volatile extracts. The dominance of margaric acid (67.34%) observed in the present study is particularly noteworthy, as this saturated fatty acid has been reported to exhibit antimicrobial and membrane-disrupting properties, which may contribute to the biological activity of the extract [13].

Table 1. Chemical distribution of compounds in the essential oil extract of *R. tinctorum*

Functional group	Percentage (%)
Fatty acid	89.29
Alkane	5.32
Terpenoid	4.57
Alkol	0.50
Aldehyde	0.30
Total	99.98

Oleic acid (8.17%) and myristic acid (5.08%) (Table 2), detected as other major fatty acids, are well-documented for their antimicrobial, anti-inflammatory, and antioxidant activities. The presence of these fatty acids suggests a potential role of the essential oil extract in inhibiting microbial growth and modulating oxidative processes. Compared to previously published studies on related plant species, the fatty acid profile of *R. tinctorum* appears to be distinct, especially in terms of the unusually high margaric acid content, indicating possible chemotypic variation or the influence of environmental and geographical factors [14, 15].

A review of the literature shows that Houari *et al.* [11], analyzed the chemical composition of the essential oil obtained from *R. tinctorum* by hydrodistillation using GC–MS and reported

cyclohexanone (88.74%) as the predominant compound. Similarly, Zhaohui (2009) [16] determined the composition of *R. tinctorum* essential oil using GC-MS and observed that theaspirane, 2-furaldehyde and benzaldehyde constitute the main compounds of the essential oil. Hoseinzadeh *et al.* [17]. investigated the essential oil composition of *R. tinctorum* using GC-MS analysis and identified bornyl acetate, asarone, sabinene hydrate acetate, ocimene, thymol, methyl

isoeugenol, neophytadiene, and methyl eugenol among the major constituents.

In the present study, terpenoids (4.57%) and alkanes (5.32%) were detected in lower proportions. These compounds are known to contribute to the aroma and biological functionality of essential oils. Terpenoids, even at low concentrations, may exhibit synergistic effects with fatty acids, thereby enhancing the overall antimicrobial or antioxidant potential of the extract [18].

Table 2. Identified compounds in the essential oil extract of *R. tinctorum*

No	RT (min)	RI	Compounds	Percentage
1	35.868	1574	Dodecanoic acid (lauric acid)	0.26
2	39.100	1720	Tetradecanal (myristaldehyde)	0.30
3	40.125	1769	Tetradecanoic acid (myristic acid)	5.08
4	41.665	1849	6,10,14-Trimethyl-2-pentadecanone	3.90
5	41.999	1867	Pentadecanoic acid (pentadecylic acid)	3.86
6	42.316	1885	1- hexadecanol (cetyl alcohol)	0.11
7	43.444	1948	9- Hexadecenoic acid	0.54
8	43.525	1952	Isophytol	0.26
9	43.906	1973	Heptadecanoic acid (margaric acid)	67.34
10	45.570	2073	13- Epi-manool	0.09
11	45.814	2088	1- Nonadecanol	0.30
12	46.730	2145	9- Octadecenoic acid (oleic acid)	8.17
13	46.793	2149	Heptadecen- (8)- carbonic acid- (1)	2.11
14	47.072	2166	Octadecanoic acid (stearic acid)	1.74
15	49.463	2321	cis-9,10-epoxy octadecanoic acid	0.19
16	50.015	2358	4,8,12,16-Tetramethyl heptadecan-4-olide	0.41
17	50.675	2401	Tetracosane	0.24
18	52.457	2492	Pentacosane	0.94
19	54.621	2601	Hexacosane	0.62
20	57.319	2690	Heptacosane	2.72
21	60.735	2802	Octacosane	0.80

RT: Retention time; RI: Retention index

CONCLUSION

In the present study, the chemical composition of *Rubia tinctorum* essential oil was characterized using GC-MS analysis, revealing fatty acids, terpenoids, and alkanes as the major constituents. It is well established that the production and accumulation of secondary metabolites in plants are influenced by environmental factors such as soil properties, mineral availability, and climatic conditions. Consequently, variations in the geographical origin and collection site of the plant may result in qualitative and quantitative differences in its secondary metabolite profile.

Overall, the chemical composition of *R. tinctorum* essential oil extract revealed in this study highlights fatty acids as the principal contributors to its phytochemical profile. The distinctive abundance of margaric acid differentiates *R. tinctorum* from other members of the genus and underscores its potential for further pharmacological and functional investigations. These results provide a scientific basis for future studies focusing on the biological activities and possible industrial or pharmaceutical applications of *R. tinctorum* essential oil.

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