Evaluation the organic acid, tocopherol and phenolic profiles of *Dracaena cinnabari* resin extracts obtained by different solvent extraction

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ABSTRACT Many plants such as *Dracaena cinnabari* have been used in traditional medicine since ancient times due to their including bioactive compounds such as phenolic compounds, tocopherols, and organic acids. The aim of present study is to determine the presence and diversity of bioactive components such as phenolic, organic acids and tocopherols of *Dracaena cinnabari* resin extracts, and to reveal which solvents can be effective in the extraction of these components and to what extent. Methanol, ethanol, chloroform, water, and vinegar were used in extraction of *Dracaena cinnabari* resin. The widest diversity was obtained with vinegar for organic acids, methanol for tocopherols, and methanol solvent for phenolic compounds. Fourteen phenolic compounds, four tocopherols and ten organic acids were detected in *Dracaena cinnabari* resin extracts, the most abundant phenolic was apigenin, while the most tocopherol was δ-tocopherol. *Dracaena cinnabari* resin extracts appear to be rich in apigenin, kaempferol, luteolin and rutin flavonoids.

KEYWORDS *Dracaena cinnabari* resin; Organic acids; Tocopherols; Phenolics; Different solvents

1. Introduction

Dragon blood is a common English name given to the red colored resins obtained from six different plant species such as *Dracaena* spp., *Daemonorops* spp., *Calamus* spp., *Pterocarpus* spp., *Eucalyptus* spp, and *Croton* spp. (Baumer and Dietemann, 2010; Al-Fatimi, 2018). *Dracaena cinnabari* Balf. f. (Dracaenaceae) is an endemic plant genus to Soqotra Island, Yemen. *Dracaena cinnabari* Balf. f. resin from Soqotra is well known as a traditional medicine with a long history. It has been traded for centuries as a medicine, and coloring agent for use in works of art (Al-Fatimi, 2018). Bioactive compounds found in medicinal and aromatic plants provide many medically important health benefits in humans (Bayram and Sagdic, 2022). *Dracaena cinnabari* Balf. f. has a wide range of medicinal uses such as antidiabetic, antihyperlipidemic (Al-Baoqai et al., 2018), haemostatic, antidiarrhetic, antiulcer (Milburn, 1984), antimicrobial (Ansari et al., 2016; Gupta and Gupta, 2011; Yehia et al., 2013), antiviral (Mothana et al., 2006), antithrombotic (Xin et al., 2011), antitumor (Al-Afifi et al., 2019b,a), anti-inflammatory (Alwashli et al., 2012; Gupta et al., 2015) and antioxidant (Gupta and Gupta, 2011; Yehia et al., 2013).

Studies on the phytochemical contents of *Dracaena cinnabari* Balf. f. show that some flavonoidins (Awthan et al., 2010), bioflavonoids (Masaoud et al., 1995a), a number of sterols and triterpenoids (Masaoud et al., 1995b) and triflavonides (Himmelreich et al., 1995) have been identified. Awthan et al. (2010) isolated 5 flavonoids from chloroform extract of dragon tree resin in a study they conducted: 4,4'-dihydroxy-2'-methoxychalcone, 4,4'-dihydroxy-2'-methoxydihydrochalcone, 7-hydroxy-3-(4-hydroxy) -4-methoxybenzyl) chroman, 7-hydroxy-3-(3-hydroxy-4-methoxybenzyl) chroman, and 2',4,4'-trihydroxychalcone. Massri (2010) isolated 3 different phenolic compounds in *Dracaena cinnabari* Balf.f. resin extracts, one of these components is glycosylated flavone, vitexin-2"-*O*-α-L-rhamnoside, one is a cinnamic acid derivative, (S)-N-trans-Coumaroyloctopamine, and the other is a new compound, trans-feruloyl-2"-hexacaric acid lactone. Himmelreich et al. (1995) isolated damalachawini, a new triflavonoid containing two deoxotetrahydrochalcone and one flavane, from the resin of the dragon blood plant (*Dracaena cinnabari*) by NMR spectroscopy.

Özcan et al. (2021) reported that in the conventional solvent extraction with distilled water of purple basil, total phenolic, flavonoid, anthocyanin contents and antioxidant activity increased with increase in temperature and time. The researchers of the stated study stated that this situation can be explained by easily the penetration of the solvent into the plant material by means of increase of temperature and time and resulted in a higher mass transfer rate.

Although there is no wide study on the phenolic, organic acid and tocopherol composition of *Dracaena cinnabari* Balf. f., there is a study in which these phytochemicals, except for tocopherol were determined in

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fruit of Dracaena draco in Dracaena species, which is also called dragon blood. Silva et al. (2011) detected 5 phenolic compounds (5-*O*-caffeoylquinic acid, ferulic acid, sinapic acid, 3,5-*O*-dicaffeoylquinic acid and quercetin-3-*O*-rutinoside), and 6 organic acids (oxalic acid, citricacid, L-ascorbic acid, malic acid, quinic acid and shikimic acid) in the water extracts of *Dracaena draco* tree fruits.

In this study, the effects of solvents such as methanol, ethanol, chloroform, water and vinegar on the organic acid, tocopherol, and phenolic composition distribution of *Dracaena cinnabari* (Dragon's blood) resin were evaluated, comparatively.

2. Methods

2.1 Plant material and extraction

Dracaena cinnabari resin used in our study was obtained from the island of Socotra (Yemen) and dried in the shade and ventilated room conditions without being exposed to direct sunlight. The dried resin was powdered by using an herb grinder (9000 rpm). Powdered samples were extracted with a Soxhlet extraction device using five different solvents such as methanol, ethanol, distilled water, chloroform, and grape vinegar at a ratio of 1:10 (w/v). Then, the solvents were removed with a rotary evaporator and lyophilized to obtain dry extract.

2.2 Phytochemical study

2.2.1 Organic acid analyses

Organic acids in resin extracts were analyzed by solid-liquid extraction method. For this, first, the Supelco C18 solid phase cartridge was conditioned with 3 mL of methanol and then washed with 10 mL of distilled water. One gram of extracts was homogenized by adding 10 mL of 2 % H3PO4 solution and filtered through Whatman No: 42 filter paper. 1 mL of the obtained eluate was diluted with 1 mL of extraction solution (0.01 M KH_2PO_4 solution adjusted to pH 8 was prepared to be used as an extraction solution). One mL of this solution was passed through the cartridge and the eluate was taken into a tube. The cartridge was washed with 1 mL of extraction solution. The eluates were combined and injected into a HPLC (Shimadzu, Tokyo-Japan) with an injection volume of 20 µL (Krapez et al., 2001).

2.2.2 Tocopherol analyses

Twenty mL of hexane on 0.5 g *Dracaena cinnabari* resin extracts were added and left for 1 night. The hexane mixture was filtered, and then the filtrates were taken into the balloon and the solvents were removed in the rotary evaporator. The remaining oil in the balloon was removed and injected into HPLC.

2.2.3 Phenolic profile analyses

To determined phenolic profiles of *Dracaena cinnabari* resin extracts, purification was carried out using a C18 Sep-Pac cartridge to remove sugars and organic acids from the extracts and obtain phenolics. Two mL of extract was loaded into conditioned C18 SPE cartridges (Agilent, USA) by passing water, ethyl acetate and methanol. To remove organic acids and sugars forming non-phenolic impurities, water was passed through the cartridge beforehand, and then the phenolics were separated with ethyl acetate and methanol. The ethyl acetate and methanol washes were removed on a rotary evaporator at 35 °C and then resuspended in 1 mL of methanol and then they were sent to HPLC with a volume of 20 µL by autosampler (Coklar and Akbulut, 2017).

The phenolic compounds analysis in D. cinnabari extracts was performed by a Shimadzu HPLC system (Shimadzu, Japan) equipped with DAD detector (λ_{max} = 278 nm), DGU-14A degasser, LC-10ADvp pump, SIL-ADvp auto sampler, SCL-10Avp system controller, and CTO-10Avp column oven.

2.2.4 HPLC systems

Chromatographic conditions for organic acid analyses

A Teknochroma TRACER EXTRASIL ODS(2) column (5 µ, 250×4.6 mm) (TR-016059), with an injection volume of 20 µL, wavelength 210 nm, and a mobile phase, H_3PO_4/H_2O (pH:2.2), flow rate 0.8 mL/min was employed.

Chromatographic conditions for tocopherol analyses

A Gl Sciences Inertsil SIL 100A column (5 µ, 250×4.6 mm), with an injection volume of 10 µL, wavelength Ex 210 nm-Em 330 nm, and a mobile phase, Hexane/2-propanol (98:2), flow rate 0.8 mL/min was employed.

Chromatographic conditions for phenolic analyses

An Agilent Eclipse XDB-C18 column (5 µ, 250×4.6 mm), with an injection volume of 20 µL, wavelength 278 nm, and a mobile phase, A: 3 % acetic acid, B: Methanol, flow rate 0.8 mL/min was employed.

3. Results

3.1 Organic acids profile of *Dracaena cinnabari* extracts

Organic acids are compounds found abundantly in plants, especially fruits. They have important activities such as antibacterial (Lacombe et al., 2010), anti-inflammatory, anticholinesterase (Liu et al., 2022). At the same time, with its reducing and chelating properties, ascorbic acid effectively increases non-heme iron absorption (Teucher et al., 2004).

Organic acid profiles of *Dracaena cinnabari* resin extracts are shown in Table 1. At same time, HPLC chromatogram of organic acid profile are presented in Figure 1. As seen in Table 1, 10 organic acids were isolated, with varying distribution in different extracts. The widest organic acid distribution was determined in vinegar extract (total of 9 organic acids), followed by vinegar > water > ethanol = methanol > chloroform extracts, respectively.

While the 10 organic acids detected in the vinegar extract, except for malic acid, were determined, it is thought that this distribution and the amounts being higher than the other extracts may be due to the organic acids in the grape vinegar. In terms of organic acid distribution, the widest distribution after vinegar was obtained from water extracts, and the quantitative distribution of the determined organic acids was determined as lactic acid > formic acid > tartaric acid > citric acid > oxalic acid > ascorbic acid > fumaric acid. It is seen that the organic acid composition

Table 1: Organic acids profiles of *Dracaena cinnabari* (Dragon's blood) resin extracts.

Organic acids (mg/g)	Extracts				
	Methanol	Ethanol	Water	Chloroform	Vinegar
Oxzalic acid	0.30	0.27	2.56	1.33	33.15(31.15)*
Tartaric acid	0.02	0.03	11.97	nd	19.98(9,54)
Formic acid	nd	nd	12.69	nd	23.71(10,87)
Malic acid	0.09	0.13	nd	nd	nd(nd)
Ascorbic acid	0.01	0.04	2.47	nd	1.49(nd)
Lactic acid	nd	nd	23.09	nd	95.58(85,97)
Acetic acid	0.12	0.46	nd	1.37	135.70(137,42)
Citric acid	nd	nd	11.09	nd	21.90(8,70)
Succinic acid	nd	nd	nd	nd	47.36(48,68)
Fumaric acid	nd	nd	0.09	nd	0.12(0,11)

*The values in parentheses are only the organic acid values of the grape vinegar used as solvent (mg/g DW). nd: not detected.

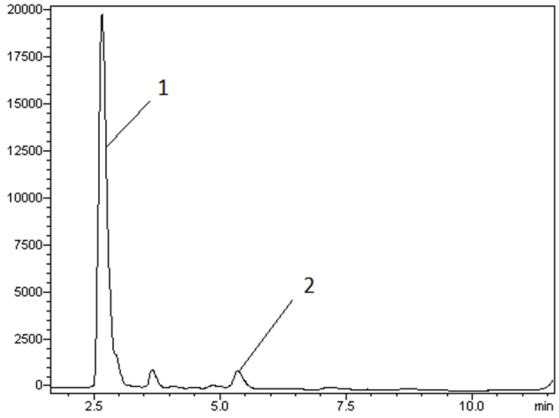

Figure 1: Organic acid chromatograms of chloroform extracts of *Dracaena cinnabari* (Dragon's blood) resin: 1. Oxalic acid, 2. Acetic acid.

of methanol and ethanol extracts are similar, and their amounts are close to each other. Only 2 organic acids, oxalic and acetic acid, were isolated in chloroform extracts, their amounts were low and close to each other in both extracts. Although it seems to be more effective in terms of obtaining more organic acids and revealing more diversity in *Dracaena cinnabari* resin extracts made with grape vinegar, this situation is understood to be due to the composition of grape vinegar.

While there is no study on the organic acid composition of the *Dracaena cinnabari* resin, in a study conducted on Dracaena dracon fruits, which are in the same plant species and have the same common name, quinic acid > malic acid > citric acid > L-ascorbic acid > oxalic acid > shikimic acids were determined (Silva et al., 2011). When the organic acid distribution obtained in our study is examined, it was understood that oxalic acid, citric acid, malic acid, and ascorbic acid were among the organic acids determined in both studies.

Table 2: Tocopherol profiles of *Dracaena cinnabari* (Dragon's blood) resin extracts.

Extracts	Tocopherols (µg/g)			
	Alpha	Beta	Gamma	Delta
Methanol	0.02	0.36	1.93	2.10
Etanol	0.23	0.15	0.20	0.27
Water	0.08	0.03	0.07	0.01
Chloroform	0.07	0.02	0.10	nd
Vinegar*	nd	0.06	0.02	nd

*No tocopherols were found in the grape vinegar used as a solvent; nd: not detected.

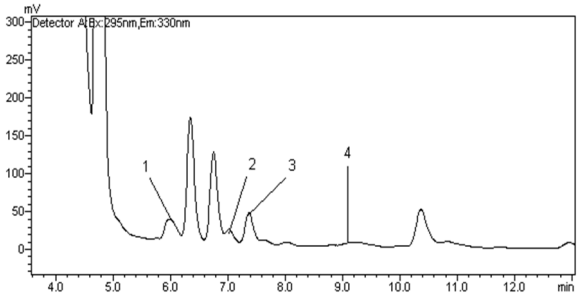

Figure 2: Tocopherol chromatograms of water extracts of *Dracaena cinnabari* (Dragon's blood) resin: 1. Alfa, 2. Beta, 3. Gamma, 4. Delta.

3.2 Tocopherols profile of *Dracaena cinnabari* extracts

Tocopherols are fat-soluble bioactive compounds and exist in 4 forms: α-, β-, γ- and δ-tocopherol. They are highly essential nutrients for human health due to their vitamin E activity and being synthesized only by photosynthetic organisms. In addition to vitamin E activities, it also has antioxidant, anticancer, cardioprotective and neuroprotective effects (Seguin et al., 2009; Coklar and Akbulut, 2019). The dietary reference intake level for vitamin E is 15 mg/day, currently based on *alpha*-tocopherol

Table 3: Phenolic profiles of *Dracaena cinnabari* (Dragon's blood) resin extracts

Phenolics (µg/g)	Extracts				
	Methanol	Ethanol	Chloroform	Water	Vinegar
Gallic acid	nd	nd	nd	80.4	60.1(42,3)*
Protocatechuic acid	nd	nd	nd	128.9	211.4(47,62)
p-Hydroxybenzoic acid	125.8	90.9	213.8	1030.7	479.4(54,2)
Chlorogenic acid	nd	nd	103.7	nd	1500.6(1524,3)
Caffeic acid	167.2	90.9	31.1	nd	139.1(130,8)
Syringic acid	107.9	80.9	14.0	965.8	113.0(nd)
Vanillic acid	45.2	35.6	97.0	nd	nd(nd)
p-Coumaric acid	nd	nd	55.2	nd	25.1(32,7)
Ferulic acid	193.9	184.1	nd	nd	nd(nd)
Sinapic acid	nd	nd	nd	841.9	122.6(nd)
Benzoic acid	909.0	nd	nd	426.0	115.6(nd)
o-Coumaric acid	25.4	24.0	41.2	nd	nd(nd)
Rutin	480.5	413.6	169.3	1312.5	88.4(15.1)
Rosmarinic acid	80.9	103.8	289.7	198.1	86.3(nd)
Cinnamic acid	151.8	113.6	124.2	338.2	73.8(2,34)
Luteolin	826.4	871.6	nd	690.1	nd(nd)
Kaempferol	5024	5638	nd	317.8	161.1(nd)
Apigenin	22830	19922	40945	4314	2190(23.9)
Total	30967	27569	42084	10435	5095

*The values in parentheses are only the phenolic compound values of the grape vinegar used as solvent (µg/g DW). nd: not detected.

only. α-tocopherol has been reported to have the highest antioxidant activity (Seguin et al., 2009). Ohkatsu et al. (2001) determined that the antioxidant activity power of tocopherols was ranked as δ- > α- > β- > γ-tocopherol, respectively.

Tocopherol contents of *Dracaena cinnabari* plant extracts in Table 2 and HPLC chromatogram of water extract are given in Fig. 2. As seen in Table 2, all four forms of tocopherols were detected in methanol, ethanol, and distilled water extracts, while α-, β- and γ- tocopherol were detected in chloroform extracts, and β and γ-tocopherol in vinegar extract. Quantitatively, the highest amounts were in methanol extracts, followed by ethanol, water, chloroform, and vinegar extracts, respectively.

The highest δ-tocopherol was found in the methanol extract with 2.10 µg/g, followed by γ- (1.93 µg/g), β- (0.36 µg/g) and α-tocopherol (0.02 µg/g), respectively.

The results obtained in this study show that methanol can be used successfully in the extraction of *Dracaena cinnabari* tocopherols and that methanol extracts can obtain extracts with high antioxidant activity due to the high content of δ-tocopherols. Indeed, Ohkatsu et al. (2001) stated in their study that the antioxidant power of δ-tocopherols is higher than other tocopherol forms.

3.3 Phenolic profile of *Dracaena cinnabari* extracts

Different solvent extracts of *Dracaena cinnabari* plant present a typical characteristic composition consisting of 18 phenolic compounds (Table 3). Fourteen of these phenolics detected in *Dracaena cinnabari* resin extracts, were phenolic acids (gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, chlorogenic acid, caffeic acid, syringic acid, vanillic acid, *o*-coumaric acid, *p*-coumaric acid, ferulic acid, sinapic acid, benzoic acid, rosmarinic acid, and cinnamic acid) and four of them were flavonoids (apigenin, kaempferol, luteolin, and rutin). To our knowledge, these phenolic compounds have been reported for the first time in *Dracaena cinnabari* resin. When looking at the phenolic compound composition according to the solvent status, the highest number of phenolic compounds was isolated in vinegar extracts. This was followed by methanol, ethanol and water and chloroform extracts, respectively. Among the phenolic compounds, apigenin appears to be the most abundant phenolic compound isolated in all extracts and quantitatively. The highest apigenin was determined as 40945 µg/g in the chloroform extract, followed by methanol > ethanol > water > vinegar extracts. In our study, apigenin, kaempferol, rutin and luteolin, which are a flavonoid, were the most quantitatively determined among the detected phenolics. The highest apigenin content was determined in chloroform extracts, the highest in routine distilled water, and the highest in ethanol extracts of kaempferol and luteolin (Table 3).

Kaczorová et al. (2021) stated that chloroform showed the best effect in revealing the diversity of phenolic compounds among petroleum ether, chloroform, ethanol, and water solvents. Coklar and Akbulut (2016) used methanol, water and methanol:water solvents for the extraction of phenolic compounds in hawthorn fruit (*Crataegus orientalis*) and determined that the extraction with methanol:water mixture was more effective in the extraction of phenolic compounds, except for gallic acid. Alothman et al. (2009) reported that the efficiency of methanol,

ethanol, water and acetone solvents in the extraction of total phenolic and total flavonoid substances from guava, banana and pineapple fruits were respectively as acetone > ethanol > water > methanol. In terms of the effect of solvents in revealing the diversity of phenolic compounds, the findings obtained in our study are similar to the results of Kaczorová et al. (2021).The widest group of naturally occurring polyphenols is flavonoids, including flavones, flavonols, flavanones, flavanols, isoflavonoides, and anthocyanidins (Salehi et al., 2019). These flavonoid group compounds act as free radical scavengers and antioxidants with antimutagenic, anti-inflammatory, antiviral, anticarcinogenic effects (Coklar and Akbulut, 2017; Shankar et al., 2017; Coklar and Akbulut, 2021).

Phenolic compounds are secondary metabolites commonly found in plants, and they are highly important bioactive compounds, especially flavonoids due to their high antioxidant capacity (Wang et al., 2008; Karimi et al., 2010). It has been stated by many researchers that phenolic compounds also have antibacterial (Mothana and Lindequist, 2005), antifungal (Al-Fatimi, 2018), anti-inflammatory (Sergent et al., 2010), antiviral (Mothana et al., 2006), anticarcinogenic (McCann et al., 2007), antitumor and cytotoxic (Al-Fatimi et al., 2005) activities. Flavonoids are widely found in plants, fruits, pollen, and flower tissues and are an important part of the diet due to their positive effects on human health. An important task of flavonoid group phenolic compounds is their strong antioxidant properties due to their highly effective scavenging properties of most oxidizing molecules such as singlet oxygen and various free radicals. Apigenin, luteolin (flavones), kaempferol (flavonols), and rutin (flavonol glycoside) are flavonoids with important functions. It is thought that the different solvent extracts of *Dracaena cinnabari* resin may have quite strong antioxidant activity since they have phenolic compounds containing these flavonoids in large quantities. Therefore, there is a need for in vivo and in vitro studies on the effects of *Dracaena cinnabari* resin extracts on human health.

4. Conclusions

Tocopherols and phenolic compounds, especially flavonoids, are among the most important bioactive components for human health. Among the phenolic compounds determined in the extracts of *Dracaena cinnabari* resin, apigenin, kaemferol, luteloin and rutin are flavonides found in abundance. Different solvents used in the extraction of *Dracaena cinnabari* resin greatly affected the profile of phenolic compounds, organic acids and tocopherol, and their individual amounts. Vinegar in terms of organic acid distribution, methanol for tocopherol distribution, and methanol, ethanol and distilled water in terms of phenolic compounds distribution and the amount of may be the most preferable.

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