

Flavonoids Content of *Dracaena cinnabari* Resin and Effects of the Aqueous Extract on Isolated Smooth Muscle Preparations, Perfused Heart, Blood Pressure and Diuresis in the Rat.

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ABSTRACT

Dracaena cinnabari, known as the dragon's blood tree, has been used for long time in the Yamani and Arabian folk medicine for many claimed ailments. Chemical analysis of the resin of *D. cinnabari* resulted in the isolation of the five flavonoid from the chloroform extract: 4,4'-dihydroxy-2'-methoxychalcone, 4,4'-dihydroxy-2-methoxydihydrochalcone, 7-hydroxy-3-(3-hydroxy-4-methoxybenzyl) chroman, 7-hydroxy-3-(4-hydroxybenzyl) chroman, and 2',4,4'-trihydroxychalcone; with the latter flavonoid being isolated for the first time from this species. The aqueous extract (AE) of the resin of *D. cinnabari*, in concentrations ranging from 10⁻⁴ to 0.03 mg/ml, caused concentration-dependent decrease of amplitude of the phasic contractions and relaxed the tone of the longitudinal segments of ileum and uterus, and urinary bladder rings. Bolus injection of AE (10⁻⁴ – 0.03 mg) increased the contractility but had no significant effect on the beating rate of the isolated perfused heart of the rat; it also had a hypotensive effect in anesthetized rats when injected in i.v. doses from 0.04 – 12 mg/kg body weight. AE (800 and 1600 mg/kg) increased significantly the rate of urine excretion in conscious rats when administered orally. These observations indicate that resin AE of *D. cinnabari* has spasmolytic, inotropic, hypotensive and diuretic effects on rats. Furthermore, the observations were discussed in relation to the claimed uses of *D. cinnabari* resin in folk medicine.

Keywords: *Dracaena cinnabari*, Dragon's blood tree, Flavonoids, Medicinal plants, Cardiovascular physiology, Diuresis.

INTRODUCTION

Dracaena cinnabari (Agavaceae) is a perennial tree that is native to Socotra Island located on the Southern coast of Yemen. The tree produces a deep red resin that has been colorfully called the Dragon's blood or the Two-brother's Blood. The dry powdered resin is often used in the Arabian Peninsula, as well as in other countries¹ as an herbal remedy for many ailments including analgesic, astringent, antiseptic, haemostatic, antiulcer and as

abortifacient if taken during the first trimester of pregnancy.^{2,3} Phytochemical studies have previously led to the isolation of a number of flavonoids, the bioflavonoid cinnabarrine, triflavonoids, sterols and triterpenoids.⁴⁻¹⁰

Few reports appeared in the literature dealing with the antioxidant activities of the ingredients of *D. cinnabari* against cytochrome P450 enzymes¹¹, with the cytotoxicity of *D. cinnabari* extract against human ECV-304 cells¹², with the antiviral activity of the methanolic extract against influenza and herpes simplex viruses¹³, and with the antibacterial and antifungal activity of the crude extract against different microorganisms.⁵ Except

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for these few reports, there is paucity of literature on the biological effects of the resin of *D. cinnabari*. Therefore, this work was carried out to further analyze the resin chemically and to test some of its biological effects against some of the claimed effects and to explore new potentials for this commonly-prescribed herbal remedy.

MATERIALS AND METHODS

Chemical analysis of the resin

The resin of *D. cinnabari* was collected from Socotra (Yemen). Eight Kgs was ground and extracted repeatedly with 95% ethanol (4x, 10 days each) and ethanol was evaporated under reduced pressure to give 1.5 Kg of a crude gummy extract. The ethanol extract was treated with 1:1 mixture of water and chloroform and the chloroform layer was separated and evaporated under reduced pressure to yield 190g. This chloroform extract was subjected to column chromatography using silica gel S (0.063-0.2 mm, Riedel-Dehaen, 700g). The column was packed in CHCl_3 and eluted with chloroform and then with chloroform- methanol mixtures of increasing polarity. The collected fractions (38; 500 ml each) were followed by TLC (silica gel plates, 0.25 mm, Merck) and grouped into four main fractions: Fraction I (1-7), fraction II (8-13), fraction III (14-30) and fraction IV (31-38). Each of the fractions I-IV was further chromatographed on a silica gel column (Kieselgel 60 HF_{254}) using benzene-ethyl acetate mixtures of increasing polarity for elution. The resulting fractions were then purified. The $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra of the isolated compounds were recorded in DMSO- d_6 on a Bruker 200 or 400 MHz instruments. The electron impact mass spectra (EIMS) were recorded on a Varian Mat 112 mass spectrometer.

Preparation of the aqueous extract of D. cinnabari:

AE was prepared by boiling 50g of the ground resin in one liter of distilled water for 15 min with continuous stirring. The solution was filtered and the filtrate was evaporated under reduced pressure at 50°C. The extract was wrapped with aluminum foil as a precaution against photooxidation. Stock solution of AE was prepared by dissolving the powdered extract in 0.9% NaCl solution

and dilutions thereof were prepared with 0.9% NaCl solution.

Protocol of the experiments

In vitro preparations

Male and female albino rats were lightly anesthetized with ether, and then sacrificed by a blow to the head. The chest and the abdominal cavities were opened to isolate the heart, a piece of the ileum, the urinary bladder, and the uterus. Two transversely-cut rings (2-3 mm each) were obtained from the middle of the urinary bladder and prepared for the recording of isometric contractions. The ileum was cleaned of excess tissue, flushed twice with physiological salt solution (PSS) and segments (1 cm long each) were prepared for isometric recording. The uterine horns were obtained from virgin females, cleaned of excess tissue, and two longitudinal segments (1 cm each) of each horn were prepared for isometric recording.

All the preparations were mounted individually in water-jacketed 10-ml glass tissue baths and connected from one end to a thread connected to a force transducer (Grass FT 03) and from the other end to a fixed component at the bottom of the tissue bath. The transducer was connected to a physiograph (Gilson Medical Electronics).

Tissues were left to equilibrate in the tissue baths for 2 hours under a tension of 1 gram and at a temperature of 37 ± 0.5 C. Tissue baths were gassed with 95% O_2 - 5% CO_2 gas mixture all through the experiment. After equilibration, a cumulative concentration-effect curve of AE was established by increasing the concentration by 3 times after the effect to the previous concentration reached a stable plateau. After the last response had plateaued, a large concentration (10^{-3}M) of papaverine was added to cause a maximum relaxation of the tissue. Responses of the tissues were expressed as percent of the maximum relaxation to papaverine.¹⁴

The isolated heart was perfused retrogradely through the aorta with aerated PSS from a reservoir located 70 cm above the heart and left to equilibrate to reach a stable plateau (10-15 minutes). Different concentrations of AE were individually injected through a needle located immediately above the aorta. The heart was then perfused

with PSS again before the injection of the next higher concentration of AE. Each response to AE was calculated as percent of the control response obtained with PSS immediately before AE administration.¹⁵

In vivo

Male albino rats (250-350 g) were anesthetized with sodium phenobarbitone (40 mg/kg body weight). The right common carotid artery was catheterized for the recording of blood pressure using Statham P23AA pressure transducer situated at the level of the heart and connected to a Gilson polygraph. The right femoral vein was also catheterized for the injection of AE. AE was injected in doses of 0.04, 0.12, 0.4, 1.2, 4, and 12 mg/kg body weight. The changes in systolic and diastolic blood pressure were recorded and expressed as percent of their respective control values obtained before AE administration.¹⁵

Diuresis

Male albino rats (250-300 g body wt.) were deprived of food for 24 hrs and of water for 30 min before the experiment. Animals were divided into 4 groups (each of 6 animals), and were administered orally with 10 ml/kg of the following treatments:

Group 1: received 0.9% NaCl solution and served as control group.

Group 2: received 800mg/kg AE.

Group 3: received 1600 mg/kg AE.

Group 4: received 40mg/kg furosemide, a known diuretic agent, and served as a positive control group.¹⁶

Animals were then individually housed into metabolic cages (North Kent Plastic Cages LTD), urine was collected continuously in graduated cylinders and the volume was recorded every two hours for a total of 24 hours.

Statistical Analysis

All data are presented as means ± SEM. Data were

analyzed with one way ANOVA. When significance was indicated, Student's *t*-test for independent samples was used to detect differences between the means. Differences were declared significant when P was < 0.05. The median effective concentration producing 50% of the maximum response (EC50) was calculated from the plot. Experimental data were analyzed by a computer fitting treatment using GraphPad Prism 5.0 software.

RESULTS

Results of chemical analysis

Fractionation of the resin resulted in the isolation and identification of five compounds. Fraction II yielded the homoisoflavan 7-hydroxy-3-(3-hydroxy-4-methoxybenzyl) chroman. Fraction III yielded the known flavonoid 7-hydroxy-3-(4-hydroxybenzyl) chroman. Fraction IV afforded the known chalcones: 4, 4'-dihydroxy-2'-methoxychalcone, 4, 4'-dihydroxy-2-methoxydihydrochalcone and 2', 4, 4'-trihydroxychalcone. The spectral data for 2', 4, 4'-trihydroxychalcone are as follows: EIMS (m/z (%): 256 (100, M⁺), 255 (92), 239 (16), 163(31), 137 (95), 120 (46), 107 (18), 91 (18).

¹H-NMR (DMSO-d₆): δ 6.27(1H, d, J= 1Hz, H-3'), 6.40 (1H, dd, J = 1 Hz and 8 Hz, H-4'), 6.85 (2H, dd, J = 8 Hz, H-3 and H-5), 7.76 (4H, m, H-2, H-6, H-α and H-6'), 8.15 (1H, d, J = 15 Hz, H-β), 8.23 and 10.20 (2H, 2s, OH-4 and OH-4'), 13.66 (1H, s, OH-2').

¹³C-NMR (DMSO-d₆): δ 103.3 (C-3'), 108.5 (C-5'), 113.6 (C-1'), 116.0 (C-3 and C-5), 118.0 (C-α), 126.9 (C-1), 131.4 (C-2 and C-6), 133.0 (C-6'), 144.5 (C-β), 160.5 (C-4), 165.1 (C-2'), 166.7 (C-4'), 192.0 (C=O).

Table 1 shows the compounds isolated and identified from *D. cinnabari* resin, and their quantities and percentages in the source plant.

Table 1: Results of chemical analysis of the resin of *Dracaena cinnabari*.

Number	Compound	Quantity (mg)	Percentage in resin (%)	Reference
1	4,4'-dihydroxy-2'-methoxychalcone	400	0.005	5
2	4,4'-dihydroxy-2-methoxydihydrochalcone	3000	0.04	4

Number	Compound	Quantity (mg)	Percentage in resin (%)	Reference
3	7-hydroxy-3-(3-hydroxy-4-methoxybenzyl)chroman	450	0.006	5
4	7-hydroxy-3-(4-hydroxybenzyl)chroman	350	0.004	4
5	2',4,4'-trihydroxychalcone	100	0.001	Current study

Effects on isolated smooth muscle preparations

Figure 1 shows that AE of *D. cinnabari* resin (10^{-5} - 3×10^{-2} mg/ml) caused a concentration-dependent decrease in the phasic contractions and in the tone of

ileum, uterus and urinary bladder. The EC_{50} of AE on these preparations were 0.023 ± 0.01 , 0.003 ± 0.02 , and 0.002 ± 0.01 mg/ml respectively.

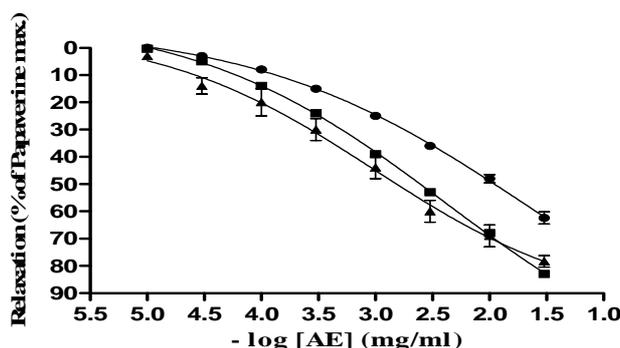


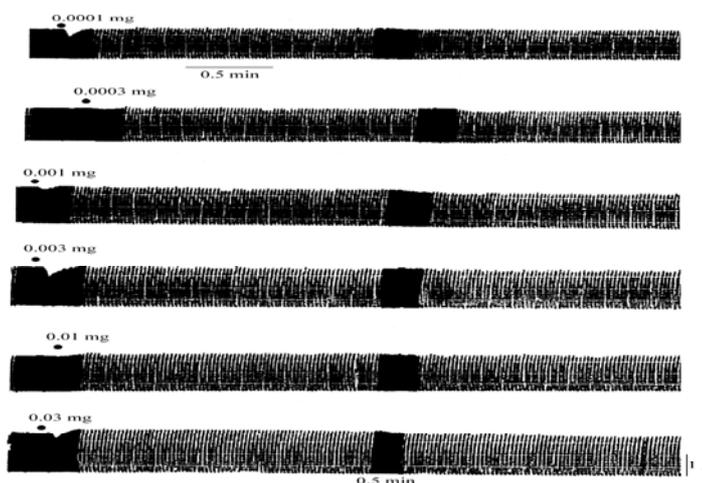
Figure 1: Cumulative concentration-response curves of aqueous extract of *Dracaena cinnabari* resin on rat isolated ileum (circles), uterus (squares) and urinary bladder (triangles). Vertical bars represent SEM of 6 experiments.

Effects on isolated perfused heart

Figures 2 and 3 show that bolus injection of AE (0.0001 – 0.03 mg/heart) increased in a dose-dependent

manner the contractility of the isolated perfused heart and had no significant effect on the heart rate.

Figure 2: Typical effects of increasing doses of aqueous extract of *Dracaena cinnabari* resin on isolated perfused heart. All traces were obtained from the same preparation. The small depression at the site of administration is an injection artifact.



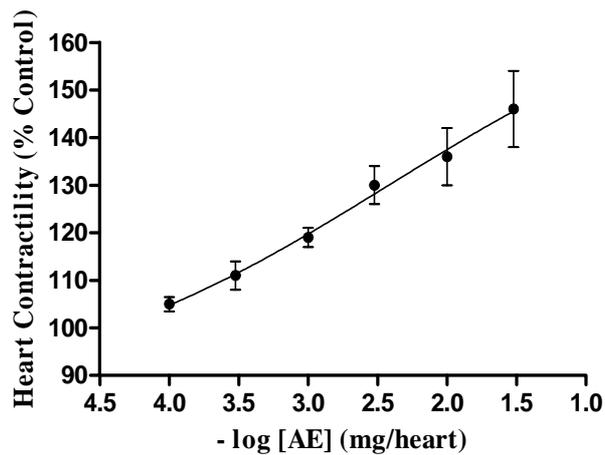


Figure 3: Cumulative concentration-response curves of aqueous extract of *Dracaena cinnabari* resin on rat isolated perfused heart contractility. Vertical bars represent SEM of 6 experiments.

Effects on blood pressure

Figure 4 shows typical responses of blood pressure of anesthetized rats to increasing doses of AE of *D. cinnabari* resin. AE caused significant decrease of both

systolic and diastolic blood pressure. Figure 5 shows the cumulative effects on systolic and diastolic blood pressure from 6 animals.

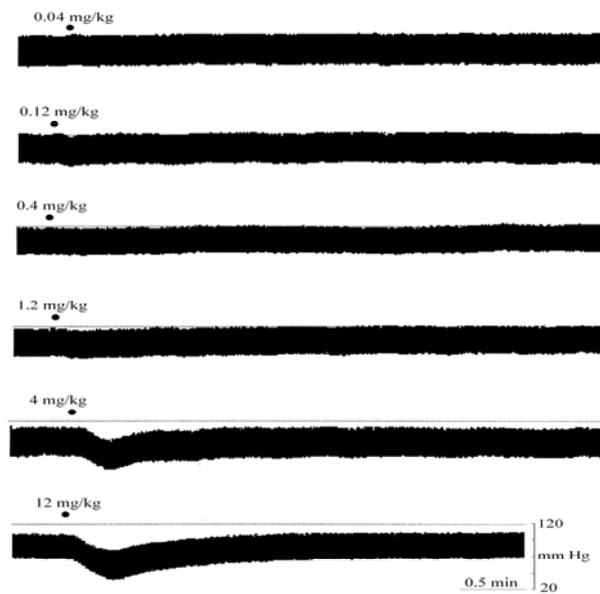


Figure 4: Typical effects of increasing doses of the aqueous extract of *D. cinnabari* resin on blood pressure of anesthetized rats. The dotted line is a reference baseline.

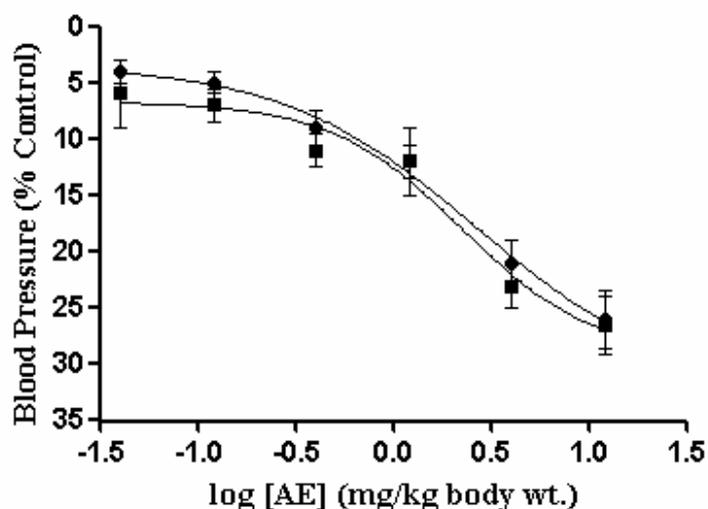


Figure 5: Cumulative effects of increasing doses of aqueous extract of *D. cinnabari* resin on systolic (triangles) and diastolic (squares) blood pressure of anesthetized rats. Values are means \pm standard error of means of 6 experiments.

Effects on diuresis

Figure 6 shows that AE, given orally at the rate of 800 or 1600 mg/kg, significantly increased urine volume excreted over a period of 24 hours when compared to a

given equal volume of 0.9%NaCl solution. This increase was only 60-68% of that induced by the standard diuretic drug furosemide.

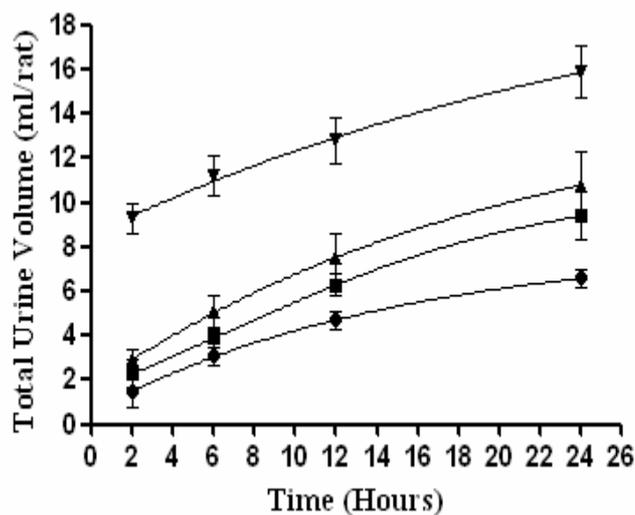


Figure 6: Effect of the aqueous extract of *D. cinnabari* resin on urine volume collected from 4 groups of rats treated with 0.9% NaCl (circles), or 800 mg/kg AE (squares), or 1600 mg/kg AE (triangles) or with furosemide (inverted triangles). Data are means \pm SEM for 6 animals.

DISCUSSION

The genus *Dracaena* seems to be rich in flavonoids. Flavonoids have been isolated from *D. cochinchensis*,¹⁸ *D. loureiri*¹⁹ and from *D. cinnabari*.^{5-8,17} The present study is in consistence with the above studies since we have shown that 5 known flavones can be isolated from the resin of *D. cinnabari*. Although the compound 2',4,4'-trihydroxychalcone is known and has been isolated previously from *D. cochinchensis*,¹⁷ it is isolated in this study for the first time from *D. cinnabari*. The five compounds were identified on the basis of their spectral data including ¹H-NMR and ¹³C-NMR and mass with the reported data.

The aqueous extract of *D. cinnabari* resin exerts a relaxant effect on rat isolated ileum, uterus and urinary bladder. This observation is consistent with the effects of other *Dracaena* species and other Dragon's blood plants. For example, *D. cochinchinensis* antagonized uterus contractions induced by stilbisterol *in vivo* in the rat.²⁰ The aqueous extract of the Dragon blood tree *Croton schiedeanus* completely relaxed contractions induced by high K in rat aortic rings in a concentration-dependent manner.²¹ The relaxant effect of AE of *D. cinnabari* resin may be attributed to the presence of flavonoids in this resin since different flavonoids have been shown to exert spasmolytic effect on smooth muscles of different preparations.²²⁻²⁶ Tests on AE of *D. cinnabari* resin showed UV active, yellow colored spots on TLC plates when sprayed with paranisaldehyde reagent, suggesting the presence of flavonoids in AE of the resin (current study; data not shown). Furthermore, chemical analysis of the resin resulted in the isolation of 5 flavonoids (Table 1 of the current study) and this is consistent with previous findings documenting the presence of flavonoids in *D. cinnabari*⁵⁻⁷ and in *D. loueiri*¹⁹ and *D. cochinchensis*.¹⁸ Flavonoids, a group of potentially water-soluble substances have been reported to have inhibitory activity against the activity of many enzyme systems such as phosphodiesterase enzyme,²⁷ thus causing relaxation of smooth muscles.

Effects of AE on the perfused heart

The present experiments show that AE of *D. cinnabari* resin increased the contractility of the isolated

perfused heart. Resins of the related Dragon's blood species *D. cochinchinensis* and *D. cambodiana* have been analyzed qualitatively and found to contain cardiac glycosides and flavonoids.²⁸ Both groups of substances are known to have positive inotropic effect on the heart. Chemical analysis of *D. cinnabari* has shown that it contains no cardiac glycosides but flavonoids are found, suggesting that the increase in heart contractility may be attributed to the presence of flavonoids. Flavonoids increase the intracellular cyclic nucleotides through inhibition of phosphodiesterases, thus inducing a positive inotropic effect.²⁹

Effect of AE on blood pressure

The present experiments showed that the AE of *D. cinnabari* resin reduced significantly both systolic and diastolic blood pressure of anesthetized rats. This effect is consistent with the effect of related Dragon's blood species since it was found that i.v. injection of the extract of *Croton schiedeanus* elicited dose-dependent decrease in rat mean arterial pressure and heart rate.²¹ This effect might have resulted from a potent vasodilatation due to relaxation of smooth muscles of blood vessels in a manner similar to its effects on other smooth muscles. AE caused an increase in heart contractility and had no remarkable effect on the heart rate; therefore, cardiac output would be expected to increase, at least, slightly. Consequently, the vasodilation produced by AE may have overcompensate for this small increase in cardiac output to produce a reasonable drop in blood pressure. Several compounds were isolated in the present study and tested for their potential hypotensive effects on anesthetized rats. For example, we have shown that the two flavonoids 4, 4'-dihydroxy-2'-methoxychalcone and 4, 4'-dihydroxy-2-methoxydihydrochalcone reduced arterial blood pressure of anesthetized rats when administered intravenously (data not shown). Flavonoids in general have almost consistently caused hypotension under different conditions using different preparations and models of study.³⁰⁻³⁵

Effect of AE on diuresis

The effect of AE on diuresis parallels the diuretic effect of the two flavonoids 4, 4'-dihydroxy-2'-

methoxychalcone and 4, 4'-dihydroxy-2-methoxydihydrochalcone in conscious rats (unpublished observations). This effect may result from the modulation of the cardiovascular reflexes by AE. For example, the hypotensive effect of AE may be related to the vasodilation or to increased glomerular filtration rate and consequently increased urinary excretion.³³ AE may also inhibit the tubular reabsorption of sodium and water, resulting in diuresis. Several flavonoids and chalcone

derivatives were found to have a diuretic effect.³⁰

In conclusion, the aqueous extract of *D. cinnabari* resin have relaxant effects on ileum, uterus and urinary bladder smooth muscle, increased heart contractility of the isolated perfused heart, reduced blood pressure of anesthetized normotensive rats and increased urine excretion in conscious rats. These effects may be taken into consideration when such resin is being used in folk medicine.

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Dracaena ennabari

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(*Dracaena cinnabari*)

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4,4'-dihydroxy-2'-methoxychalcone, 4,4'-dihydroxy-2-methoxydihydrochalcone, 7-hydroxy-3-(3-hydroxy-4-methoxybenzyl) chroman, 7-hydroxy-3-(4-hydroxybenzyl) chroman, 2',4,4'-trihydroxychalcone

/ 0.03 4- 10

0.03 4-10

/ 12 0.04

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