

Comparison of the chemical profiles and anti-platelet aggregation effects of two “Dragon's Blood” drugs used in traditional Chinese medicine

Yi, Tao; Chen, Hu-Biao; Zhao, Zhong-Zhen; Yu, Zhi-Ling; Jiang, Zhi-Hong

Published in:
Journal of Ethnopharmacology

DOI:
[10.1016/j.jep.2010.11.008](https://doi.org/10.1016/j.jep.2010.11.008)

Published: 27/01/2011

Document Version:
Peer reviewed version

[Link to publication](#)

Citation for published version (APA):
Yi, T., Chen, H.-B., Zhao, Z.-Z., Yu, Z.-L., & Jiang, Z.-H. (2011). Comparison of the chemical profiles and anti-platelet aggregation effects of two “Dragon's Blood” drugs used in traditional Chinese medicine. *Journal of Ethnopharmacology*, 133(2), 796-802. <https://doi.org/10.1016/j.jep.2010.11.008>

General rights

Copyright and intellectual property rights for the publications made accessible in HKBU Scholars are retained by the authors and/or other copyright owners. In addition to the restrictions prescribed by the Copyright Ordinance of Hong Kong, all users and readers must also observe the following terms of use:

- Users may download and print one copy of any publication from HKBU Scholars for the purpose of private study or research
- Users cannot further distribute the material or use it for any profit-making activity or commercial gain
- To share publications in HKBU Scholars with others, users are welcome to freely distribute the permanent publication URLs

1
2
3 **Comparison of the Chemical Profiles and Anti-platelet Aggregation Effects of Two**
4
5 **“Dragon’s Blood” Drugs Used in Traditional Chinese Medicine**
6
7

8 Tao Yi, Hu-Biao Chen*, Zhong-Zhen Zhao, Zhi-Ling Yu, Zhi-Hong Jiang
9

10
11
12
13
14
15 **Affiliation**
16

17 School of Chinese Medicine, Hong Kong Baptist University, Hong Kong Special Administrative Region, P. R. China
18
19
20
21
22

23 Corresponding author. Tel.: +852 3411 2060; fax: +852 3411 2461.
24

25 *E-mail address:* hbchen@hkbu.edu.hk (H.B. Chen).
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3 **ABSTRACT**
4

5 *Ethnopharmacological relevance:*

6 “Dragon’s Blood” has been used as a medicine since ancient times by many cultures. In traditional
7 Chinese medicine, the resin obtained from *Daemonorops draco* (RDD) and the resin from *Dracaena*
8 *cochinchinensis* (RDC) are equally prescribed as “Dragon’s Blood” for facilitating blood circulation.
9
10

11
12
13 *Aim of the study:*

14 To verify the traditional efficacy and elucidate the mechanism, the present study compared the
15 chemical profiles and the pharmacological effects of two species of “Dragon’s Blood” mainly used in
16 China.
17
18

19
20
21 *Materials and methods:*

22 A UPLC-MS fingerprinting method was developed to compare the chemical profiles of the two
23 medicines. The anti-platelet aggregation effects of the two medicines induced by arachidonic acid (AA)
24 were investigated.
25
26

27
28
29 *Results:*

30 The chemical profiles of these two species of “Dragon’s Blood” were significantly different. The
31 characteristic constituents were found to be: flavanes in RDD and stilbenes in RDC. In the *in vivo*
32 platelet inhibition test, performed with the dose of 200 mg/kg on rats, the peak inhibitory effects of
33 RDD and RDC were 35.8% and 27.6%, respectively, compared with the control group. With the *in*
34 *vitro* concentrations of 0.2, 0.4 and 0.8 mg/ml, RDD exerted significant inhibition of aggregation by
35 18.7%, 20.0%, and 61.6%, respectively, and RDC exerted significant inhibition of aggregation by
36 13.3%, 20.2%, and 31.6%, respectively.
37
38
39
40

41
42
43 *Conclusion:*

44 The fingerprinting method used here is suitable for distinguishing them. All pharmacological tests
45 indicated that RDD was more potent than RDC against platelet aggregation.
46
47

48
49
50 *Keywords:*

51 Dragon’s Blood; *Daemonorops draco*; *Dracaena cochinchinensis*; chemical profiles; anti-platelet
52 aggregation
53
54
55
56
57
58
59
60
61
62
63
64
65

1. Introduction

“Dragon’s Blood” is a deep red resin obtained from species of *Dracaena* (Dracaenaceae), *Daemonorops* (Palmaceae), *Croton* (Euphorbiaceae) and *Pterocarpus* genera (Fabaceae) (Pearson and Prendergast, 2001). It has been used as a famous ethnomedicine since ancient times by many cultures (Gupta et al., 2008). Having a reputation for facilitating blood circulation and dispersing blood stasis, in traditional Chinese medicine, this resinous medicine is commonly prescribed to invigorate blood circulation for the treatment of traumatic injuries, blood stasis and pain (Chinese Pharmacopoeia Commission, 2005; Commission of Chinese Materia Medica, 1999).

The historical uses of “Dragon’s Blood” can be traced back to ancient Greece and ancient Arabia (Angiosperm Phylogeny Group, 1974). In A.D. 77-78, “Dragon’s Blood” was firstly listed in *De Materia Medica* by the Greek doctor Dioscorides (A.D. 40-90); it is believed that the botanical source of the drug at that time was several species of the *Dracaena* genus, such as *D. draco* and *D. cinnabari*, distributed in the Soktra Island of Yemen (Mabberley, 1998; Milburn, 1984). Later, “Dragon’s Blood” was not only very famous in Europe, but also in China, reaching the Far East via the “Silk Road” during the Sui and Tang dynasties (A.D. 581-907). With the development of maritime trade between China and Southeast Asia from Ming dynasty (A.D. 1368-1644), the resin secreted from the fruit of *Daemonorops draco* (Willd.) Blume, a plant indigenous to Indonesia and Malaysia, was shipped to China and used as “Dragon’s Blood” (Xie, 1989). Due to the higher price of resin from *Daemonorops draco*, the search for alternative sources has been ongoing. Until 1972, a new plant source of “Dragon’s Blood”, *Dracaena cochinchinensis* (Lour.) S.C. Chen, was found in Yunnan province of China. Since then, the resin extracted from stems of *Dracaena cochinchinensis* with ethanol has been used as “Dragon’s Blood” (Cai and Xu, 1979.). Subsequently, *D. cambodiana* Pierre ex Gagnep., another species of the *Dracaena* genus distributed in Hainan province of China, was also studied for obtaining “Dragon’s Blood”; however, rarity blocked industrial-scale production (Zheng et al., 2003). In summary, two species are currently the primary sources for the widely used ethnomedicine “Dragon’s Blood” in China; these are the resin obtained from *Daemonorops draco* (RDD) and the resin from *Dracaena cochinchinensis* (RDC) (Fig. 1).

Insert Fig. 1 here

There are two problems in the current use of “Dragon’s Blood” resins from these two species: 1) distinguishing one from the other; and 2) determining whether they are in fact equally effective in clinical use. Distinguishing the two is important because, while the resins derived from the two look similar, they differ significantly in price. RDD is much more expensive. Hence, there are many

1
2
3 attempts to make RDC appear to be RDD, and sell it at higher prices (Chen, 2005). Unfortunately,
4 attempts to distinguish between the two medicines using empirical methods have met with little success
5 (Ren et al., 2006; Wang, 2005), and the identification carried out by spectrophotometry and thin layer
6 chromatography can not provide the exact information of characteristic compounds (Song and Hu,
7 2009). Recently, a HPLC method based on flavylum chromophores as species markers has been
8 reported to identify three species of “Dragon’s Blood” commonly traded in Europe; however, RDC was
9 not one of the research objectives (Sousa et al., 2008), and RDC may be unsuitable for this method due
10 to absence of flavylum chromophores (Gupta et al., 2008). To solve this problem, it is desirable to
11 develop a novel method based on chemical identification to distinguish the two resinous medicines
12 used in China. At the same time, we don’t actually know whether the two species are equally effective
13 as drugs. Comparisons of their pharmacological potencies based on the clinical indications are needed.
14 Laboratory studies suggest that “Dragon’s Blood” species exert their clinical effects by inhibiting blood
15 platelet aggregation (Commission of Chinese Materia Medica, 1999; Lu et al., 2003); thus measuring
16 anti-platelet aggregation is an accepted test for evaluating their clinical effects (Jackson, 2007).

17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Aware of these two fundamental problems, in recent years, our research group has focused on the research on “Dragon’s Blood”. In our previous study, we reported the microscopic features and major constituents of *Dracaena* plants, one genus of the original plants for obtaining “Dragon’s Blood” (Fan et al., 2008; Fan et al., 2009). Thus, in the present follow-up study, we further differentiated two “Dragon’s Blood” medicines using chemical fingerprinting method, and compared their inhibitory effects on rat platelet aggregation induced by arachidonic acid. The results revealed that the developed protocol could unambiguously authenticate the two medicines, and that the characteristic constituents are flavanes in RDD and stilbenes in RDC. Anti-platelet aggregation tests showed that the inhibitory effects of RDD were more potent than those of RDC. These results suggest that the two drugs should be distinguished when sold and used.

2. Materials and methods

2.1 Materials

The sources of the RDD and RDC samples are listed in Table 2 and Table 3. Identity of these samples was confirmed by Dr. Hu-Biao Chen, and voucher specimens were deposited in the School of Chinese, Hong Kong Baptist University (JK-01 for RDD and GC-05 for RDC).

2.2 Chemicals and Reagents

The standard compounds of loureirin A, loureirin B and resveratrol were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Other

1
2
3 standard compounds were isolated by our laboratory with a purity of more than 98%, and their
4 chemical structures were elucidated by comparing with literature data of ^1H and ^{13}C NMR (Mu et al.,
5 1999; Shen et al., 2007; Tsai, 1993; Tu et al., 2003). Their chemical structures are shown in Fig. 2.
6
7 Acetonitrile and methanol of chromatography grade were purchased from Lab-scan (Bangkok,
8 Thailand). Formic acid and ethanol of analytical grade were purchased from Merck (Darmstadt,
9 Germany).

13
14 *Insert Fig. 2 here*

16 2.3 Sample extraction

17
18 The sample powder (0.1 kg) was extracted with 95% ethanol by means of sonication at room
19 temperature for 30 min. The operations were repeated until the extract became colorless. The combined
20 extracts were evaporated to remove ethanol at reduced pressure in a rotary evaporator (50 °C). RDD
21 extracts (yield 75.6-82.5%, w/w) and RDC extracts (yield 93.7-98.6%, w/w) were thus obtained.

24 2.4 UPLC-PDA-ESI/MS analysis

25
26 A Waters AcquityTM ultra-performance liquid chromatography (UPLC) system (Waters Corp.,
27 Milford, USA) with photo diode array (PDA) detector, was hyphenated to a Bruker MicrOTOFQ
28 system by an electrospray ionization (ESI) interface (Bruker Daltonics, Bremen, Germany) for
29 chromatographic and spectrometric (MS) analysis. The chromatographic separation was carried out on
30 a Waters BEH C₁₈ column (1.7 μm, 2.1 × 100 mm, Waters Corp.) with a VanGuardTM pre-column
31 (BEH, C₁₈, 1.7 μm, 2.1 × 5 mm). The mobile phase consisted of 0.1% formic acid in water (A) and
32 0.1% formic acid in acetonitrile (B) using a gradient program of 15-20% (B) in 0-8 min and 20-68% (B)
33 in 8-30 min. The solvent flow rate was 0.3 ml/min, the column temperature was set to 40 °C and the
34 detection wavelength was 280 nm. The conditions of MS analysis in the positive ion mode were as
35 follows: drying gas (nitrogen), flow rate, 8 l/min; gas temperature, 180 °C; scan range, 50-1000 m/z;
36 end plate offset voltage, -500 V; capillary voltage, 4500 V; nebulizer press, 2.5 Bar.

37
38 For the chemical comparison, about 0.1 g of each extract was dissolved in 25 ml of methanol, and
39 then filtered through a syringe filter (0.2 μm) to obtain sample solution. The stock solutions of standard
40 compounds (0.5 mg/ml) were respectively prepared in methanol and diluted appropriately before use.
41 An aliquot of 1 μl of sample solution and standard solution was injected into the UPLC-PDA-ESI/MS
42 system for analysis, respectively.

56 2.5 Comparison of the chemical profiles by fingerprinting method

57
58 Relative retention time (RRT) and relative peak area (RPA) of each characteristic peak related to
59 the reference peak were calculated for quantitative expression of the chemical properties in the
60
61
62
63
64
65

1
2
3 chromatographic pattern. RRT was calculated according to the following equation:
4

$$5 \quad RRT = \frac{\text{Retention time of selected peak}}{\text{Retention time of reference peak}},$$

6
7
8 while relative peak area (RPA) was calculated as follows:
9

$$10 \quad RPA = \frac{\text{Area of selected peak}}{\text{Area of reference peak}}.$$

11
12
13 The relative standard deviation (RSD) was calculated to assess the stability of the present protocol as
14 well as the test samples.
15
16
17

18 *2.6 Comparison of the anti-platelet aggregation effects*

19 *2.6.1 Animals*

20
21 Male SD rats weighing 200-220 g were purchased from the Laboratory Animal Services Center, the
22 Chinese University of Hong Kong. All experimental protocols were approved by the Committee on the
23 Use of Human & Animal Subjects in Teaching and Research of Hong Kong Baptist University, in
24 accordance with the Animals Ordinance (Department of Health, Hong Kong).
25
26
27
28

29 *2.6.2 Preparation of reagents*

30
31 Arachidonic acid (AA), aspirin, carboxymethyl cellulose (CMC) and dimethyl sulfoxide (DMSO)
32 were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). AA was dissolved in a small
33 volume of absolute ethanol and then adjusted with 0.9% normal saline to obtain the final concentration
34 of 20 mM.
35
36
37

38 *2.6.3 In vivo platelet aggregation assay*

39
40 For the determination of *in vivo* inhibitory effects on rat platelet aggregation, the model described
41 by Lau and Yao (Lau et al., 2009; Yao et al., 2008) was employed with modifications. The dried
42 extracts of RDD and RDC were suspended in 1% (w/v) aqueous CMC for oral administration to
43 animals. SD rats were weighed and randomized into eight groups of six animals each, namely a control
44 group (1% CMC-treated), a reference drug group (20 mg/kg of aspirin-treated), and three groups each
45 (receiving 50, 100, or 200 mg/kg of medicine) for RDD treatment and RDC treatment. The test agents
46 were orally administered to the rats for seven consecutive days. These rats received no food but water
47 *ad libitum* and were killed 2 h after the last delivery. Citrated blood was centrifuged at $160 \times g$ for 10
48 min and the supernatant was removed as platelet rich plasma (PRP). Platelet aggregation assay was
49 performed with a 560 CA Chrono-Log aggregometer (Chrono-Log, Havertown, PA, USA) according to
50 the optical method (Born, 1962). Rat PRP (490 μ l) was incubated at 37 °C for 3 min in the
51 aggregometer while stirring at 1000 rpm, and platelet aggregation was induced by the addition of 10 μ l
52
53
54
55
56
57
58
59
60
61
62
63
64
65

1
2
3 of AA solution. Changes in light transmission were monitored for 5 min after stimulation with AA.

4 5 2.6.4 *In vitro* platelet aggregation assay

6
7 *In vitro* platelet aggregation assay with modifications was performed as previously described
8 (Jeenapongsa et al., 2003; Yun et al., 2001). The extracts of two medicines were dissolved in DMSO
9 and further diluted with 0.9% normal saline to various concentrations for bioassay. Rat PRP (470 µl)
10 from control group was incubated at 37 °C for 3 min in the aggregometer while stirring at 1000 rpm,
11 and a fixed amount (20 µl) of the test solutions or saline was added, and the mixture was incubated at
12 37 °C for 3 min. After incubation, platelet aggregation was induced by the addition of 10 µl of AA
13 solution. Changes in light transmission were recorded for 5 min after stimulation with the AA. Normal
14 saline was used in control test, while aspirin of 0.1 mg/ml was served as the reference drug.
15
16
17
18
19
20

21 2.6.5 *Statistical analysis*

22
23 The aggregation is expressed as percent of inhibition (I) using the following equation:

$$24 \quad I(\%) = \frac{A - B}{A} \times 100,$$

25
26
27 where A is maximal aggregation of the control test and B is maximal aggregation of drug-treated PRP.
28 Values obtained from experiments were expressed as mean ± S.E.M and further analyzed using
29 one-way ANOVA followed by Dunnett test for multiple comparisons, with the level of significance
30 chosen as $P < 0.05$.
31
32
33
34
35
36

37 **3. Results and discussion**

38 3.1 *Optimization of the separation conditions*

39
40 The choice of UPLC columns was conducted by separating samples on different chromatographic
41 columns, and the best separation was observed with BEH C₁₈ column using gradient elution. Column
42 temperature was screened at 35, 40 and 45 °C, and the optimized column temperature is 40 °C.
43 Acetonitrile was preferred over methanol as the mobile phase because of its use resulted in an
44 improved separation and a significantly reduced column pressure. Peak resolution and peak purity were
45 continuously monitored by PDA and MS detector. In order to obtain satisfactory peak resolution and
46 peak purity in chromatograms, various linear gradients of water and acetonitrile at a flow rate of 0.3
47 ml/min were further optimized to well separate all of the characteristic peaks within 30 min. By
48 comparing the chromatograms of the samples acquired at different wavelengths within 190-500 nm, it
49 was found that 280 nm could well represent the chemical profiles of the two medicines. The typical
50 UPLC-PDA chromatograms are shown in Fig. 3.
51
52
53
54
55
56
57
58
59
60

61 *Insert Fig. 3 here*

1
2
3
4 *3.2 Online ESI/MS identification of the characteristic constituents*
5

6 During the optimization of mass spectrometric conditions, the positive ion mode was found to be
7 more sensitive, and most components exhibited their quasi-molecular ions $[M+H]^+$ and $[M+Na]^+$.
8

9 *Insert Table 1 here*
10

11
12 Based on the obtained m/z values, UV spectra and comparison with standard compounds, ten peaks
13 were unambiguously identified as resveratrol (1), 7,4'-dihydroxyflavone (2), 4,6-dihydroxy-2-
14 methoxy-3-methyl-dihydrochalcone (3), 4,6-dihydroxy-2-methoxy-3-methylchalcone (4), (2S)-5,7-
15 dihydroxy-dihydroflavone (5), loureirin A (6), loureirin B (7), pterostilbene (8), (2S)-5-methoxyflavan-
16 7-ol (9) and (2S)-5-methoxy-6-methylflavan-7-ol (10). The chromatographic and spectrometric data of
17 the identified compounds are listed in Table 1. The results show that both medicines contain flavonoids,
18 including flavones (peak 2 in RDC and peak 5 in RDD) and chalcones (peak 3, 4 in RDD and peak 6, 7
19 in RDC). On the other hand, the results also demonstrate that the characteristic constituents are:
20 flavanes in RDD (peak 9, 10), and stilbenes in RDC (peak 1, 8). Thus, it was suitable to choose peak 1
21 and peak 10 as the reference peaks of RDC and RDD, respectively, for fingerprint calculation.
22
23
24
25
26
27
28
29
30
31

32 *3.3 Comparison of the chemical profiles of two medicines by UPLC fingerprinting method*
33

34 The UPLC-PDA chromatograms were overlapped to visually compare the chemical profiles of two
35 medicines (Fig. 3). Peak 1 and peak 10, as the characteristic peaks of RDC and RDD respectively, were
36 chosen as the reference peaks for fingerprint calculation. The relative retention time (RRT) and relative
37 peak area (RPA) generated with respect to the reference peak were used to distinguish two medicines
38 and assess the consistency from batch-to-batch (Table 2 and Table 3).
39
40
41
42

43 *Insert Table 2 and Table 3 here*
44

45 From the chromatograms and the calculating results, two findings emerge. Firstly, the chemical
46 profiles of two medicines differed greatly; there were virtually no matched peaks between RDC and
47 RDD in the chromatograms. This result not only directly proved that the chemical compositions of
48 RDC and RDD were different, but also indicated that the present method could distinguish two
49 medicines unambiguously.
50
51
52
53

54 Secondly, in all the 10 batches of medicines including 5 RDC samples and 5 RDD samples, the
55 relative standard deviation (RSD) values of RRT were less than 0.139%, and the RSD values of RPA
56 were reported in the range of within 12.88-36.34% except the reference peak. The present results
57 demonstrated that the profiles were generally consistent within species, although the peak intensities
58
59
60
61
62
63
64
65

1
2
3 differed significantly. Therefore, the statistical model of RRT is superior to that of RPA because of the
4 stability, and comparison with RRT parameters is recommended to distinguish two species of
5 “Dragon’s Blood”. In short, both findings suggest that the present fingerprinting method is suitable for
6 authentication purposes among species and for consistency assessment within species of the two
7
8
9
10 medicines.

11 12 13 *3.4 Comparison of the in vivo anti-platelet aggregation effects*

14
15 Among platelet functional tests, light transmission aggregometry (LTA) is still regarded as the
16 classic standard for measuring platelet function (Harrison et al., 2007). Besides platelet rich plasma
17 (PRP), washed platelet (WP) is also the common test sample by LTA (Amrani et al., 2009; Mekhfi et al.,
18 2004). In our preliminary tests, WP was found to be easier to lose aggregability than PRP, thus, PRP
19 was used in this study. The representative samples were chosen to conduct the pharmacological tests,
20 and the administrative dosage for animal experiments was converted from the clinical dosage for
21 human beings (Chinese Pharmacopoeia Commission, 2005; Commission of Chinese Materia Medica,
22 1999).

23
24 The *in vivo* suppressive effects of the two medicines on AA-induced rat platelet aggregation are
25 shown in Fig. 4. The extracts of the two medicines exhibited varying degrees of anti-platelet
26 aggregation activity. RDD extract administered orally at doses of 50, 100 and 200 mg/kg showed
27 significant dose-dependent reduction of the platelet aggregation, and the percentage platelet inhibition
28 was 8.5%, 25.3% and 35.8%, respectively. Oral administration of RDC extract (50, 100 and 200 mg/kg)
29 resulted in a significant inhibition of aggregation by 20.5%, 13.6% and 27.6%, respectively. Compared
30 to RDC, RDD showed higher percentages against *in vivo* platelet aggregation. The peak inhibitory
31 effects of RDD and RDC (35.8% and 27.6%, respectively) were recorded with the dose of 200 mg/kg,
32 compared with the control group.

33
34
35
36
37
38
39
40
41
42
43
44
45
46
47 *Insert Fig. 4 here*

48 49 *3.5 Comparison of the in vitro anti-platelet aggregation effects*

50
51 To corroborate the findings from the *in vivo* platelet inhibition studies of “Dragon’s Blood”, *in vitro*
52 platelet aggregation assays were also performed, and the results are shown in Fig. 5.

53
54 As shown in Fig. 5, RDD and RDC both showed dose-dependent inhibitory activity towards
55 AA-induced rat platelet aggregation. At the *in vitro* concentrations of 0.2, 0.4 and 0.8 mg/ml, RDD
56 resulted in a significant inhibition of aggregation by 18.7%, 20.0%, and 61.6%, respectively, and RDC
57 resulted in a significant inhibition of aggregation by 13.3%, 20.2%, and 31.6%, respectively. *In vivo*
58
59
60
61
62
63
64
65

1
2
3 platelet inhibition induced by AA, RDD showed significantly higher percentage than RDC, and this
4 trend was almost consistent at different concentrations.
5
6

7 *Insert Fig. 5 here*
8

9 Platelets are known to aggregate in the presence of a number of different reagents. For optical
10 aggregometry, AA is one of the common used aggregating agents. When added to blood, AA is
11 converted to prostaglandin endoperoxides in the presence of cyclooxygenases (COX), and then these
12 endoperoxides are converted to thromboxane A2 (TXA2) in the presence of TXA2 synthase. TXA2 and
13 the endoperoxides, including prostaglandin G2 (PGG2), prostaglandin H2 (PGH2), are further
14 responsible for platelet aggregation (Xu et al., 2002). Thus, in the present study, we used AA with
15 definite aggregating mechanisms to investigate the anti-platelet mechanism of “Dragon’s Blood”. From
16 the results of *in vitro* tests, both RDD and RDC showed inhibitory potency against the aggregation
17 induced by AA, which suggests that the two medicines exhibit their anti-platelet effects through
18 inactivating COX or TXA2 synthase, and then blocking the AA metabolic pathway.
19
20

21 From the results of chemical identification shown in Fig. 3 and Table 1, flavonoids including
22 flavones, chalcones and flavanes, are found to be the common or characteristic compounds in RDD and
23 RDC. The anti-platelet effects of flavonoids constituents with various types have also been confirmed
24 by many reports in the literature (Afifi and Aburjai 2004; Jong and Wu 1988; Ko et al., 2004; Wang et
25 al., 2010), and COX inhibition and blockage of AA metabolism are important pathways by which they
26 work (Tsai et al., 2003; Wu et al., 2007). Particularly, it has been reported that the underlying
27 mechanism for anti-platelet activity of (2S)-5-methoxy-6-methylflavan-7-ol (peak 10) was related to
28 inhibition of TXA2 formation via the inhibition of COX (Tsai et al., 1998). Based on these findings, we
29 believe that the flavonoids are responsible for the clinical effects of “Dragon’s Blood”. Flavonoids in
30 the two medicines might be cyclooxygenase inhibitors, serving to block the AA metabolic pathway, and
31 then producing anti-platelet aggregation. Moreover, the difference in inhibitory potencies of RDD and
32 RDC, shown in the *in vivo* and *in vitro* aggregation tests, would contribute to the various types,
33 abundances and bioavailabilities of flavonoids in the two medicines. For the further study of “Dragon’s
34 Blood”, it would have been useful to also test the *in vitro* pharmacological assay of the isolated
35 compounds.
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56

57 **4. Conclusion**

58 Of the existing studies on “Dragon’s Blood”, the present study is the first report to compare the
59 chemical profiles and the pharmacological effects of two species of “Dragon’s Blood”, including the
60
61
62
63
64
65

1
2
3 resins obtained from *Daemonorops draco* (RDD) and *Dracaena cochinchinensis* (RDC). The results of
4 chemical analysis demonstrated that the characteristic constituents of the two medicines are flavanes in
5 RDD and stilbenes in RDC. Moreover, the relative retention time (RRT) and relative peak area (RPA)
6 were calculated, and shown to differ. Thus, the present fingerprinting method is suitable for
7 authentication purposes among species and consistency assessment within species of the two
8 medicines.
9

10
11
12
13
14 The present study also investigated the *in vivo* and *in vitro* anti-platelet aggregation effects of the
15 two medicines. Results verified the traditional efficacy of “Dragon’s Blood” for facilitating blood
16 circulation. Both RDD and RDC exhibited anti-platelet aggregation activities, although their potencies
17 differed. This novel finding suggests that it is necessary to discriminate between the two medicines
18 when using them. Meanwhile, this result of pharmacological comparison also highlighted that it was
19 indispensable to develop a chemical fingerprinting method for the authentication of the two medicines,
20 which are similar in appearance but different in potency.
21
22
23
24
25
26
27

28 **Acknowledgements**

29
30 This research was funded by the Faculty Research Grant of Hong Kong Baptist University
31 (FRG/08-09/II-52).
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

References

- Afifi, F.U., Aburjai, T., 2004. Antiplatelet activity of *Varthemia iphionoides*. *Fitoterapia* 75, 629–633.
- Amrani, S., Harnafi, H., Gadi, D., Mekhfi, H., Legssyer, A., Aziz, M., Martin-Nizard, F., Bosca, L., 2009. Vasorelaxant and anti-platelet aggregation effects of aqueous *Ocimum basilicum* extract. *Journal of Ethnopharmacology* 125, 157–162.
- Angiosperm Phylogeny Group, 1974. *Annals of the Missouri Botanical Garden* 85, 531.
- Born, G.V.R., 1962. Aggregation of blood platelets by adenosine diphosphated and its reversal. *Nature* 194, 927–929.
- Cai, X.T., Xu, Z.F., 1979. Study on the plant origin of Chinese Dragon's Blood. *Acta Botanica Yunnanica* 1, 1–9.
- Chen, J.G., 2005. Authentication of Dragon's Blood. *Journal of Practical Traditional Chinese Medicine* 21, 767.
- Chinese Pharmacopoeia Commission, 2010. *Pharmacopoeia of the People's Republic of China*, vol. 1. Chemical Industry Publishing Press, Beijing, China, pp. 133.
- Commission of Chinese Materia Medica, 1999. *Materia Medica of China*, vol. 8. Shanghai Scientific and Technical Publishers, Shanghai, China, pp. 455–458.
- Fan, L.L., Tu, P.F., Chen, H.B., Cai, S.Q., 2009. Simultaneous quantification of five major constituents in stems of *Dracaena* plants and related medicinal preparations from China and Vietnam by HPLC–DAD. *Biomedical Chromatography* 23, 1191–1200.
- Fan, L.L., Tu, P.F., He, J.X., Chen, H.B., Cai, S.Q., 2008. Microscopical study of original plant of Chinese drug “Dragon's Blood” *Dracaena cochinchinensis* and distribution and constituents detection of its resin. *China Journal of Chinese Materia Medica* 33, 1112–1117.
- Gupta, D., Bleakley, B., Gupta R.K., 2008. Dragon's Blood: Botany, chemistry and therapeutic uses. *Journal of Ethnopharmacology* 115, 361–380.
- Harrison, P., Frelinger III, A.L., Furman, M.I., Michelson, A.D., 2007. Measuring antiplatelet drug effects in the laboratory. *Thrombosis Research* 120, 323–336.
- Jackson, S.P., 2007. The growing complexity of platelet aggregation. *Blood* 109, 5087–5095.
- Jeenapongsa, R., Yoovathaworn, K., Sriwatanakul, K.M., Pongprayoon, U., Sriwatanakul, K., 2003. Anti-inflammatory activity of (*E*)-1-(3,4-dimethoxyphenyl) butadiene from *Zingiber cassumunar* Roxb. *Journal of Ethnopharmacology* 87, 143–148.
- Jong, T.T., Wu, T.S., 1988. Highly oxygenated flavonoids from *Melicope triphylla*. *Phytochemistry* 28, 245–246.
- Ko, H.H., Hsieh, H.K., Liu, C.T., Lin, H.C., Teng, C.M., Lin, C.N., 2004. Structure-activity relationship studies on chalcone derivatives: Potent inhibition of platelet aggregation. *Journal of Pharmacy and Pharmacology* 56, 1333–1337.
- Lau, A.J., Toh, D.F., Chua, T.K., Pang, Y.K., Woo, S.O., Koh, H.L., 2009. Antiplatelet and anticoagulant effects of *Panax notoginseng*: Comparison of raw and steamed *Panax notoginseng* with *Panax ginseng* and *Panax quinquefolium*. *Journal of Ethnopharmacology* 125, 380–386.
- Lu, H., Teng, J.B., Wu, H.E., 2003. Advances in the studies of Dragon's Blood produced in China. *Chinese Journal of Ethnomedicine and Ethnopharmacy* 12, 264–267.
- Mabberley, D.J., 1998. *The Plant Book*. Cambridge University Press (rev. edn1998).
- Mekhfi, H., Haouari, M.E., Legssyer, A., Bnouham, M., Aziz, M., Atmani, F., Remmal, A., Ziyat, A., 2004. Platelet anti-aggregant property of some Moroccan medicinal plants. *Journal of Ethnopharmacology* 94, 317–322.
- Milburn, M., 1984. Dragon's Blood in East & West Africa, Arabia and the Canary Islands. *Africa* 39, 486–493.
- Mu, Q., Li, C.M., He, Y.N., Sun, H.D., Zheng, H.L., Lu, Y., Zheng, Q.T., Wu, N., Jiang, R.W., 1999. Three new styrylpyrones from *Goniothalamus leiocarpus*. *Chinese Chemical Letters* 10, 135–138.
- Pearson, J., Prendergast, D.V., 2001. Collections corner: *Daemonrops*, *Dracaena* and other Dragons Blood. *Economic Botany* 55, 474–477.
- Ren, J.Y., Zhang, Q.B., Jiang, W.H., 2006. Identification study on Dragon's Blood and their adulterations. *Information on Traditional Chinese Medicine* 23, 31–32.
- Shen, C.C., Tsai, S.Y., Wei, S.L., Wang, S.T., Shieh, B.J., Chen, C.C., 2007. Flavonoids isolated from *Draconis Resina*. *Natural Product Research* 21, 377–380.
- Song, Q.L., Hu, S.F., 2009. Identification of *Sanguis Draconis* and *Resina Draconis* by TLC and UV. *China Pharmacist* 12, 1314–1315.

- 1
2
3 Sousa, M.M., Melo, M.J., Parola, A.J., Seixas de Melo, J.S., Catarino, F., Pina, F., Cook, F.E., Simmonds, M.S.,
4 Lopes, J.A., 2008. Flavylum chromophores as species markers for dragon's blood resins from *Dracaena*
5 and *Daemonorops* trees. *Journal of Chromatography* 1209, 153–161.
- 6 Tsai, S.Y., 1993. Studies on the constituents of *Phyllodium pulchellum* and Draconis Resina. Master thesis.
7 Chung Yuan Christian University, Taiwan.
- 8 Tsai, W.J., Hsieh, H.T., Chen, C.C., Chen, C.F., Kuo, Y.C., Chen, C.F., 1998. Characterization of the antiplatelet
9 effects of (2S)-5-methoxy-6-methylflavan-7-ol from Draconis Resina. *European Journal of Pharmacology*
10 346, 103–110.
- 11 Tsai, W.J., Lin, Y.L., Ho, Y.C., Kuo, Y.C., 2003. Inhibition of cyclic AMP phosphodiesterase and blockage of
12 arachidonate metabolism by antiplatelet principles from the seed hulls of *Arachis hypogaea* L. *Chinese*
13 *Pharmaceutical Journal* (Taipei, Taiwan) 55, 335–345.
- 14 Tu, P.F., Tao, J., Hu, Y.Q., Zhao, M.B., 2003. Flavones from the wood *Dracaena conchinchinensis*. *Chinese*
15 *Journal of Natural Medicines* 1, 27–29.
- 16 Wang, A.M., Zhang, F.K., Huang, L.F., Yin, X.P., Li, H.F., Wang, Q.Y., Zeng, Z.W., Xie, T., 2010. New progress
17 in biocatalysis and biotransformation of flavonoids. *Journal of Medicinal Plants Research* 4, 847–856.
- 18 Wang, Z.X., 2005. Authentication of Sanguis Draconis, Resina Draconis and their adulterations. *Lishizhen*
19 *Medicine and Materia Medica Research* 16, 269.
- 20 Wu, C.M., Wu, S.C., Chung, W.J., Lin, H.C., Chen, K.T., Chen, Y.C., Hsu, M.F., Yang, J.M., Wang, J.P., Lin,
21 C.N., 2007. Antiplatelet effect and selective binding to cyclooxygenase (COX) by molecular docking
22 analysis of flavonoids and lignans. *International Journal of Molecular Sciences* 8, 830–841.
- 23 Xie, Z.W., 1989. Study on the origin of Dragon's Blood. *Journal of Chinese Medicinal Materials* 12, 40–43.
- 24 Xu, S.Y., Bian, R.L., Chen, X., 2002. Methodology of Pharmacological Experiment. People's Medical
25 Publishing House, Beijing, China, pp. 1276–1289.
- 26 Yao, Y., Wu, W.Y., Liu, A.H., Deng, S.S., Bi, K.S., Liu, X., Guo, D.A., 2008. Interaction of salvianolic acids and
27 notoginsenosides in inhibition of ADP-induced platelet aggregation. *The American Journal of Chinese*
28 *Medicine* 36, 313–328.
- 29 Yun, Y.P., Do, J.H., Ko, S.R., Ryu, S.Y., Kim, J.H., Song, H.C., Park, Y.D., Ahn, K.S., Kim, S.H., 2001. Effects
30 of Korean red ginseng and its mixed prescription on the high molecular weight dextran-induced blood
31 stasis in rats and human platelet aggregation. *Journal of Ethnopharmacology* 77, 259–264.
- 32 Zheng, Q.A., Chen, J.T., Zhang, Y.J., Yang, C.R., 2003. Origin and diffusion of Dragon's Blood, a famous folk
33 herb. *Acta Botanica Yunnanica* S14, 102–107.
- 34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

Legends for Tables

Table 1

Chromatographic and spectrometric data of the identified compounds in the UPLC chromatograms.

Table 2

Fingerprint data of the characteristic peaks in 5 RDC samples.

Table 3

Fingerprint data of the characteristic peaks in 5 RDD samples.

Table 1

Chromatographic and spectrometric data of the identified compounds in the UPLC chromatograms.

Peak	Retention time (min)	Identification	Other peak (<i>m/z</i>)	[<i>M</i> +H] ⁺ (<i>m/z</i>)	[<i>M</i> +Na] ⁺ (<i>m/z</i>)	λ max (nm)	Chemical type
1	7.0	Resveratrol	135	229	251	214, 305	Stilbene
2	7.6	7,4'-Dihydroxyflavone	-	255	277	230, 331	Flavone
3	17.0	4,6-Dihydroxy-2-methoxy-3-methylchalcone	167, 269	287	309	243, 281	Chalcone
4	17.6	4,6-Dihydroxy-2-methoxy-3-methylchalcone	105, 267	285	307	262, 377	Chalcone
5	18.1	(2 <i>S</i>)-5,7-Dihydroxy-dihydroflavone	131, 153	257	279	210, 289	Flavone
6	18.4	Loureirin A	137, 151	287	309	220, 278	Chalcone
7	18.7	Loureirin B	137, 181	317	339	222, 277	Chalcone
8	20.4	Pterostilbene	-	257	-	219, 306	Stilbene
9	21.0	(2 <i>S</i>)-5-Methoxyflavan-7-ol	117, 153	257	279	230, 271	Flavane
10	21.7	(2 <i>S</i>)-5-Methoxy-6-methylflavan-7-ol	117, 167	271	293	232, 283	Flavane

Table 2

Fingerprint data of the characteristic peaks in 5 RDC samples.

Samples	Source	Peak 1 (R ₁)		Peak 2		Peak 6		Peak 7		Peak 8	
		RRT	RPA	RRT	RPA	RRT	RPA	RRT	RPA	RRT	RPA
RDC-1	Mengla, Yunnan (2009)	1.0000	1.0000	1.0788	0.4533	2.6099	2.2531	2.652	0.9392	2.8847	1.5784
RDC-2	Nanning, Guangxi (2004)	1.0000	1.0000	1.0784	0.6253	2.6099	1.1243	2.652	1.0515	2.8847	2.6871
RDC-3	Jinghong, Yunnan (2008)	1.0000	1.0000	1.0786	0.5448	2.6094	1.5837	2.6517	1.3427	2.8841	2.9374
RDC-4	Mengla, Yunnan (2008)	1.0000	1.0000	1.0782	0.5165	2.6088	2.2112	2.6509	1.3634	2.8833	2.7913
RDC-5	Pu'er, Yunnan (2009)	1.0000	1.0000	1.0799	0.3983	2.6172	0.9811	2.6598	0.9298	2.8931	2.2271
	Mean	1.0000	1.0000	1.0788	0.5076	2.6110	1.6307	2.6533	1.1253	2.8860	2.4443
	RSD (%)	0.000	0.00	0.062	17.12	0.133	36.34	0.138	18.97	0.139	22.59

RDC-1 to RDC-5: Resins obtained from *Dracaena cochinchinensis*.Peak 1 (R₁): Peak 1 was chosen as the reference peak to match the selected peaks in RDC samples.

Table 3

Fingerprint data of the characteristic peaks in 5 RDD samples.

Samples	Source	Peak 3		Peak 4		Peak 5		Peak 9		Peak 10 (R ₂)	
		RRT	RPA	RRT	RPA	RRT	RPA	RRT	RPA	RRT	RPA
RDD-1	Tianjin (2010)	0.7810	0.3923	0.8091	0.8659	0.8321	0.4418	0.9666	0.7449	1.0000	1.0000
RDD-2	Beijing (2006)	0.7808	0.5287	0.8090	0.7353	0.8320	0.5385	0.9667	0.8899	1.0000	1.0000
RDD-3	Chengdu, Sicuan (2008)	0.7818	0.2716	0.8091	0.5312	0.8319	0.6131	0.9667	0.9493	1.0000	1.0000
RDD-4	Hong Kong (2008)	0.7810	0.2567	0.8092	0.3922	0.8322	0.5546	0.9667	0.7978	1.0000	1.0000
RDD-5	Hong Kong (2009)	0.7809	0.2837	0.8092	0.5518	0.8319	0.6385	0.9667	1.0272	1.0000	1.0000
	Mean	0.7811	0.3466	0.8091	0.6153	0.8320	0.5573	0.9667	0.8818	1.0000	1.0000
	RSD (%)	0.051	33.18	0.010	30.20	0.016	13.73	0.005	12.88	0.000	0.00

RDD-1 to RDD-5: Resins obtained from *Daemonorops draco*.Peak 10 (R₂): Peak 10 was chosen as the reference peak to match the selected peaks in RDD samples.

Legends for Figures

Fig. 1. Photos of the resin obtained from *Daemonorops draco* (RDD, the upper row) and the resin from *Dracaena cochinchinensis* (RDC, the lower row).

Fig. 2. Chemical structures of the identified compounds in the UPLC chromatograms.

Fig. 3. Typical UPLC-PDA chromatograms of RDC and RDD samples.

Fig. 4. *In vivo* inhibitory effects of RDD and RDC on rats platelet aggregation induced by AA. Values of edema shown are mean \pm S.E.M. ($n=6$). * $p < 0.05$; ** $p < 0.01$ vs. control group.

Fig. 5. *In vitro* inhibitory effects of RDD and RDC on rats platelet aggregation induced by AA. Values of edema shown are mean \pm S.E.M. ($n=6$). * $p < 0.05$; ** $p < 0.01$ vs. control group.



Fig. 1. Photos of the resin obtained from *Daemonorops draco* (RDD, the upper row) and the resin from *Dracaena cochinchinensis* (RDC, the lower row).

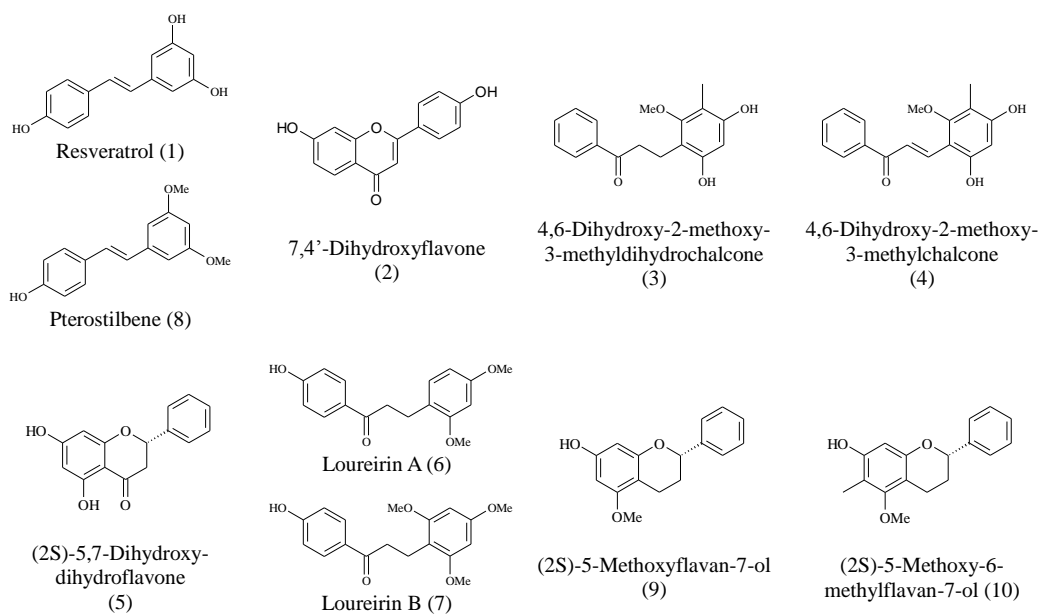


Fig. 2. Chemical structures of the identified compounds in the UPLC chromatograms.

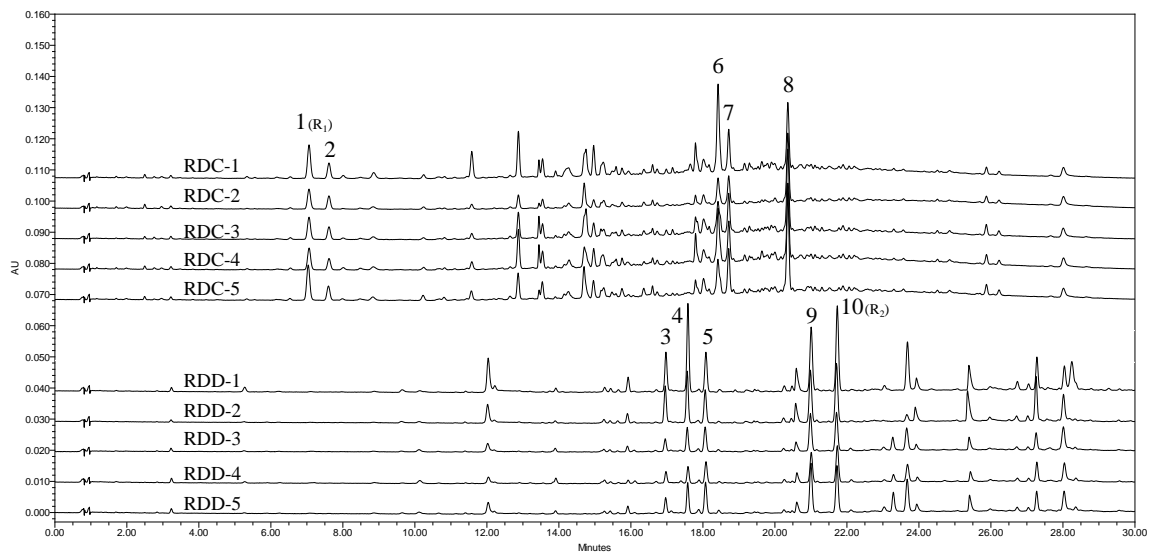


Fig. 3. Typical UPLC-PDA chromatograms of RDC and RDD samples.

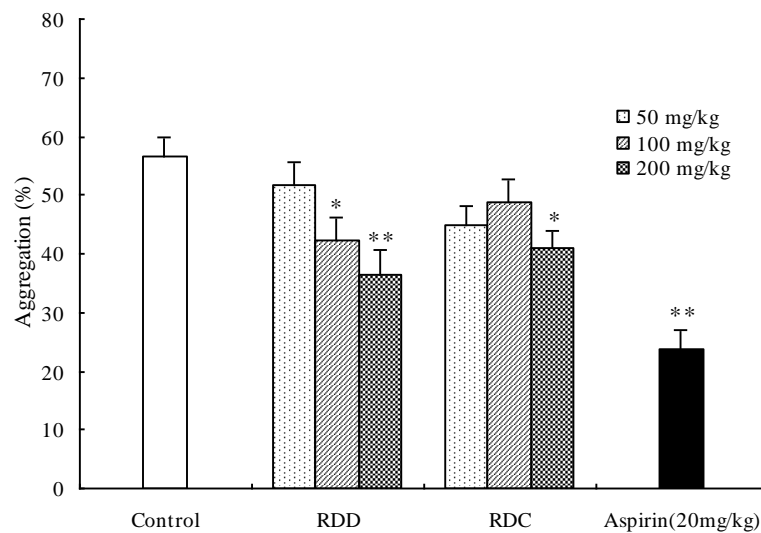


Fig. 4. *In vivo* inhibitory effects of RDD and RDC on rats platelet aggregation induced by AA.

Values of edema shown are mean \pm S.E.M. ($n=6$).

* $p < 0.05$; ** $p < 0.01$ vs. control group.

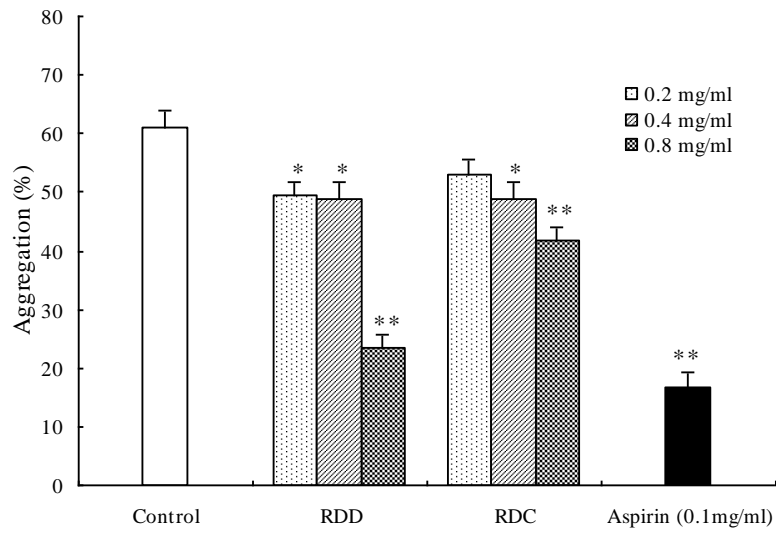


Fig. 5. *In vitro* inhibitory effects of RDD and RDC on rats platelet aggregation induced by AA.

Values of edema shown are mean \pm S.E.M. ($n=6$).

* $p < 0.05$; ** $p < 0.01$ vs. control group.