**Study on the inhibitory effect of various combinations of   
Drug Combination-6 on SARS-CoV-2 3CLPro and PLP**

**1.** **Preparation technology of Drug Combination-6 stipulated by Chinese Pharmacopeia**

**1.1** **Drug composition**

**Table 1． Formula ratio of Drug Combination-6 in   
Chinese Pharmacopeia 2020 edition**

|  |  |  |  |
| --- | --- | --- | --- |
| Herb | Weight (gram) | Herb | Weight (gram) |
| Forsythia Suspensa | 255 | Chrysanthemum | 255 |
| Ephedra | 85 | Ginkgo | 85 |
| Rhizoma Anemarrhenae | 255 | Golden Cypress | 255 |
| Nightshade | 255 | Flos Daturae | 255 |
| Perilla | 85 | China Rose | 51 |
| Radix Peucedani | 85 | Affine Cudweed | 7.5 |
| Glechoma longituba | 85 |  |  |

**1.2** **Preparation Process**

Perilla was extracted with volatile oil by distillation in water. The volatile oil was collected, the aqueous extract was filtered, and set aside. Forsythia Suspensa, Ephedra, Flos Daturae, and China Rose were extracted twice with 70% ethanol, the first time for 2h and the second time for 1.5h. Extracts were filtered, combined. Ethanol was recovered and set aside. Chrysanthemum, Rhizoma Anemarrhenae, Golden Cypress, Nightshade, Glechoma longituba, and Radix Peucedani were decocted in water until boiling, added to Ginkgo and decocted twice. The first time for 1.5h and the second time for 1h. The decoction was filtered. Filtrate was combined and added to an aqueous solution of Perilla oil extracted and ready for use, concentrated to a relative density of 1.10-1.15 (60°C). Ethanol was added to bring the alcohol content to 70%, refrigerated at 4°C for 24h, filtered and the filtrate was recovered as ethanol. The filtrate was recovered from the ethanol and combined with the alcohol extract of Forsythia Suspensa and the other four flavors above, concentrated to a relative density of 1.15~1.20 (60°C) and spray dried. Mixed with the appropriate amount of starch, made granules, dried, sieved and sift out the appropriate amount of fine powder. Dissolve Affine Cudweed, Perilla volatile oil with the appropriate amount of ethanol. Sprayed into the fine powder, mixed with the above particles, and closed the capsule for 30min, and made 1000 capsules.

**2. Research design of dismantling formula of Drug Combination-6**

The capsules have been recombined according to the "monarch-minister- assistant-conductant drug" formula. There are four combinations. (1) Monarch drugs: Chrysanthemum, Fructus Forsythiae; (2) Monarch- minister drugs: Chrysanthemum, Fructus Forsythiae, Perilla, Ginkgo, and Rhizoma Anemarrhenae; (3) Monarch-minister-assistant drugs: Chrysanthemum, Fructus Forsythiae, Perilla, Ginkgo, Rhizoma Anemarrhenae, Golden Cypress, China Rose , Herba Menthae Haplocalycis, Perilla, Flos Daturae, Bisset Wood Fern, and Radix Peucedani; (4) Monarch-minister-assistant-conductant drugs: Chrysanthemum, Fructus Forsythiae, Perilla, Ginkgo, Rhizoma Anemarrhenae, Golden Cypress, China Rose , Herba Menthae Haplocalycis, Perilla, Flos Daturae, Bisset Wood Fern, Radix Peucedani, and Glechoma longituba.

**3. Preparation of dismantled combination of Drug Combination-6**

Different split recipe compositions were prepared according to the preparation method specified in the pharmacopeia. The dosage of herbs for each split formula combination is shown in Table 2.

**Table 2. The dosage of herbs in each combination of the dismantled formula of the Drug Combination-6**

|  |  |  |
| --- | --- | --- |
| Dismantling parties | Herbs | Herb dosage (g) |
| Monarch drugs | Fructus Forsythiae | 5.1 |
| Chrysanthemum | 5.1 |
| Minister drugs | Roasted Ephedra | 1.7 |
| Ginkgo | 1.7 |
| Rhizoma Anemarrhenae | 5.1 |
| Assistant drugs | Golden Cypress | 5.1 |
| Male Fern Rhizome | 5.1 |
| Flos Daturae | 5.1 |
| Perilla | 1.7 |
| China Rose | 1.02 |
| Radix Peucedani | 1.7 |
| Mint Brain | 0.15 |
| Conductant drug | Glechoma longituba | 1.7 |

The preparation process is as follows.

**3.1 Preparation of monarch drugs**

Take Fructus Forsythiae powder 5.1 g in a round bottom flask, add 51.0 mL (10 times the amount) of 70% ethanol and soak for 30min, extract twice, the first time for 2h, and the second time for 1.5h. Extracts were filtered, combined, and concentrated. Take 5.1g of Chrysanthemum powder in a round bottom flask, add 51.0mL (10 times the amount) of water, soak for 30min, heat to boiling, decoct twice, the first time for 1.5h, the second time for 1h. The decoction was filtered. The filtrate was combined and concentrated to a relative density of 1.10~1.15 (60°C), add ethanol to reach 70% alcohol content, refrigerated at 4°C for 24h, filter through, and recover the ethanol from the filtrate. The ethanol was recovered from the filtrate and combined, concentrated and lyophilized with the above extract.

**3.2** **Preparation of monarch-minister drugs**

Fructus Forsythiae 5.1g, scorched Perilla 1.7g in a round bottom flask, add 68.0mL (10 times the amount) of 70% ethanol soak for 30min, extract twice, the first 2h, the second 1.5h. The extract was filtered, combined, concentrated. Take 5.1g of Chrysanthemum powder and 5.1g of Rhizoma Anemarrhenae in a round bottom flask, add 119.0mL (10 times the amount) of water and soak for 30min, heat to boiling, add 1.7g of Ginkgo, decoct twice, the first time for 1.5h, the second time for 1h. The decoction was filtered and concentrated. The filtrate was combined and concentrated. The ethanol was recovered from the filtrate and combined, concentrated, and lyophilized with the above extract.

**3.3** **Preparation of monarch-minister-assistant drugs**

Fructus Forsythiae 5.1g, sizzling Perilla 1.7g, Flos Daturae 5.1g, and China Rose 1.02g were put in a round bottom flask, soaked in 129.2ml (10 times) 70% ethanol for 30 min, and extracted for two times, 2 h for the first time and 1.5 h for the second time. The extracted solution was filtered, combined and concentrated. Chrysanthemum powder 5.1g, Rhizoma Anemarrhenae 5.1g, Golden Cypress powder 5.1g, Sheep Horse Bisset Wood Fern powder 5.1g, Radix Peucedani powder 1.7g, Perilla 1.7g were put in a round bottom flask, adding 255.0mL (10 times the amount) of water and soaking for 30min, heat to boiling. Add Ginkgo 1.7g, decoct twice, the first time for 1.5h, the second time for 1h. The decoction was filtered. The filtrate was combined and concentrated to a relative density of 1.10~1.15 (60°C). The filtrate was combined, concentrated to a relative density of 1.10~1.15 (60°C), added with ethanol to reach 70% alcohol content, refrigerated at 4°C for 24h, and filtered. Combine with the alcohol extract of Fructus Forsythiae and the other four flavors above, concentrate to a relative density of 1.15~1.20 (60°C), add Perilla volatile oil lyophilized, and reserve.

**3.4 Preparation of monarch-minister-assistant-conductant** **drugs**

Fructus Forsythiae 5.1g, sizzling Perilla 1.7g, Flos Daturae 5.1g, and China Rose 1.02g were put in a round bottom flask, added 129.2mL (10 times the amount) of 70% ethanol and soaked. Extracts were filtered, combined, and concentrated. Chrysanthemum powder 5.1g, Rhizoma Anemarrhenae 5.1g, Golden Cypress powder 5.1g, Sheep Horse Bisset Wood Fern powder 5.1g, Radix Peucedani powder 1.7g, Glechoma longituba 1.7g, and Perilla 1.7g were placed in a round bottom flask, soaked in 272.0mL (10 times the amount) of water for 30min, heated to boiling, added Ginkgo 1.7g, decocted twice. The first time for 1.5h, the second time for 1h. The decoction was filtered, the filtrate was combined and concentrated to a relative. The filtrate was combined and concentrated to a relative density of 1.10~1.15 (60°C). Ethanol was added to bring the alcohol content to 70%, refrigerated at 4°C for 24h, and filtered. Combine with the alcohol extract of Fructus Forsythiae and the other four flavors mentioned above, concentrate to a relative density of 1.15~1.20 (60°C), add Perilla volatile oil and lyophilize.

**4. Extraction results (weight of lyophilized powder)**

**Table 3. Extract quality of each dismantled combination of Drug Combination-6**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Split-square combination | Dosing amount  (gram) | | Extract mass  (gram) | Percentage  (%) |
| Monarch | | 10.2 | 4.17 | 40.88% |
| Monarch-minister | | 18.7 | 6.35 | 33.96% |
| Monarch-minister-assistant | | 38.57 | 12.87 | 33.37% |
| Monarch-minister-assistant-conductant | | 40.27 | 14.71 | 36.53% |

**5.** **Inhibition of SARS-CoV-2 3CLpro by various combinations of dismantled formulae of Drug Combination-6**

**5.1** **Preparation of extracts**

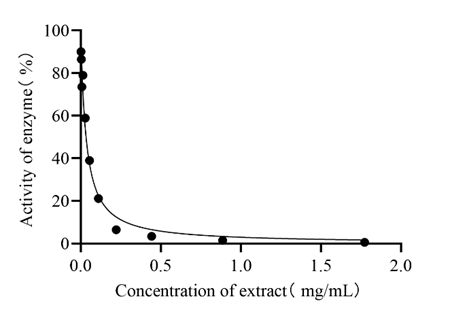
Extracts of each dismantled combination, extracted according to the pharmacopeia, were dissolved in DMSO, 100μL dispensed and stored at -20°C. The specific drugs configuration was as follows: (1) Monarch drugs extract: Lyophilized powder 0.0993 g dissolved in 2.8 mL DMSO, 17000 g, centrifuged at room temperature for 5 min, removed the precipitation. Thus, the concentration of Monarch drugs extract was 35.4643mg/mL. (2) Monarch-minister drugs extract: Lyophilized powder 0.0988g was dissolved in 3.7mL DMSO at 17000g and clarified by centrifugation at room temperature for 5min. Thus, the concentration of monarch-minister drugs extract was 26.7027 mg/mL. (3) Monarch-minister-assistant drugs extract: Lyophilized powder 0.1022g dissolved in 6.9mL DMSO 17000g and centrifuged at room temperature for 5min to remove the precipitate. Thus, the concentration of monarch-minister-assistant drugs extract was 14.8116mg/mL. (4) Monarch-minister-assistant-conductant drugs extract: Lyophilized powder 0.1099g was dissolved in 5.4mL of DMSO at 17000g. The precipitate was removed by centrifugation at room temperature for 5min. Thus, the concentration of monarch-minister-assistant-conductant drugs extract was 20.3519mg/mL.

**5.2** **Determination of** **IC50 of extracts from each combination of the dismantled formula of Drug Combination-6 against SARS-CoV-2 3CLpro**

Extracts of each dismantled combination of Drug Combination-6 were diluted 2-fold with DMSO for a total of 11 extract concentrations. Prokaryotic expressed and purified by nickel column SARS-CoV-2 3CLpro and the fluorescent substrate Dabcyl-KTSAVLQSGFRKME-Edans (synthesized by Nanjing Peptide Industry) solubilized with Mili-Q were diluted with enzyme activation buffer (50 mM Tris-HCl (pH 7.3), 1mM EDTA). SARS-CoV-2 3CLpro 40uL (final concentration of 100nM) was incubated with 10uL of extracts of each dismantled combination of Drug Combination-6 at different concentrations for 30min at room temperature, and then the fluorescent substrate (final concentration of 25μM) was added for immediate fluorescence intensity detection. The assay conditions are as follows. Excitation: 336/20, emission: 490/20, interval 30s, shaking plate 0.01s for 1h, and gain value 100. To exclude the effect of different concentrations of extracts of each dismantled combination of Drug Combination-6 on the fluorescence intensity, a control without SARS-CoV-2 3CLpro was set.

The calculation method is as follows. Enzyme activity efficiency = [(fluorescence signal at the endpoint of detection of each combination of extracts of different concentrations of Drug Combination-6 - fluorescence signal at the beginning of detection) - (fluorescence signal at the endpoint of detection of each combination of extracts of different concentrations of Drug Combination-6 without SARS-CoV-2 3CLpro under the same conditions - fluorescence signal at the beginning of detection)]/[( fluorescence signal at the detection endpoint of positive control DMSO - fluorescence signal at the detection starting point) – (no SARS-CoV-2 3CLPro DMSO detection endpoint fluorescence signal under the same conditions - detection starting point fluorescence signal)] ×100. The concentrations and the corresponding enzymatic activity efficiencies of SARS-CoV-2 3CLpro for each extract of the dismantled combination of Drug Combination-6 were imported into GraphPad Prism. The IC50 of the drugs was fitted by choosing Inhibitor vs. normalized response-Variable slope.

**Experimental results**



**Fig. 1**

As shown in Fig. 1, the activity of SARS-CoV-2 3CLpro gradually decreased as the concentration of monarch drugs extract increased, indicating that monarch drugs extract had an inhibitory effect on SARS-CoV-2 3CLpro with an IC50 of 0.0333 mg/ml.



**Fig. 2**

As shown in Fig. 2, the activity of SARS-CoV-2 3CLpro gradually decreased as the concentration of monarch-minister drugs extracts increased, indicating that monarch-minister drugs extracts had an inhibitory effect on SARS-CoV-2 3CLpro. SARS-CoV-2 3CLpro was inhibited with an IC50 of 0.0374 mg/ml.



**Fig. 3**

As shown in Fig. 3, the activity of SARS-CoV-2 3CLpro gradually decreased as the concentration of monarch-minister-assistant drugs extracts increased, indicating that monarch-minister-assistant drugs extracts had an inhibitory effect on SARS-CoV-2 3CLpro. SARS-CoV-2 3CLpro was inhibited with an IC50 of 0.0795 mg/ml.



**Fig. 4**

As shown in Fig. 4, the activity of SARS-CoV-2 3CLpro gradually decreased as the concentration of monarch-minister-assistant-conductant drugs extract increased, indicating that monarch-minister-assistant-conductant drugs extract had an inhibitory effect on SARS-CoV-2 3CLpro with an IC50 of 0.1028 mg/ml.

**5.3** **Extrapolation of** **IC50 for SARS-CoV-2 3CLpro from the original herbs of each dismantled combination of Drug Combination-6**

The IC50 of extracts of each split formula of Drug Combination-6 against SARS-CoV-2 3CLpro can be deduced from the IC50 of the raw herbs of each split formula of Drug Combination-6 against SARS-CoV-2 3CLpro. The formula for the deduction is as follows. Drug Combination-6 each prescription extract to SARS-CoV-2 3CLpro IC50 × each combination of the dosage / the quality of the extract of each combination.

**Table 4 IC50 of various combinations of the dismantled formula of Drug Combination-6**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Split-square combination | Dosing amount (gram) | | | IC50 of raw herbs (mg/mL) | | Extract mass (gram) | | IC50 of extracts (mg/mL) |
| Monarch | | 10.2 | 0.0815 | | 4.17 | | 0.0333 | |
| Monarch-minister | | 18.7 | 0.1101 | | 6.35 | | 0.0374 | |
| Monarch-minister-assistant | | 38.57 | 0.2383 | | 12.87 | | 0.0795 | |
| Monarch-minister-assistant-conductant | | 40.27 | 0.2814 | | 14.71 | | 0.1028 | |

As shown in Table 4, the IC50 of monarch drugs, monarch-minister drugs, monarch-minister-assistant drugs, and monarch-minister-assistant-conductant drugs for SARS-COV-23 3CLpro were 0.0815mg/mL, 0.1101mg/mL and 0.2383mg /mL, 0.2814 mg/mL, respectively. Therefore, the inhibitory effect of monarch drugs, monarch-minister drugs, monarch-minister-assistant drugs, and monarch-minister-assistant-conductant drugs on SARS-COV-23 3CLpro was gradually weakened.

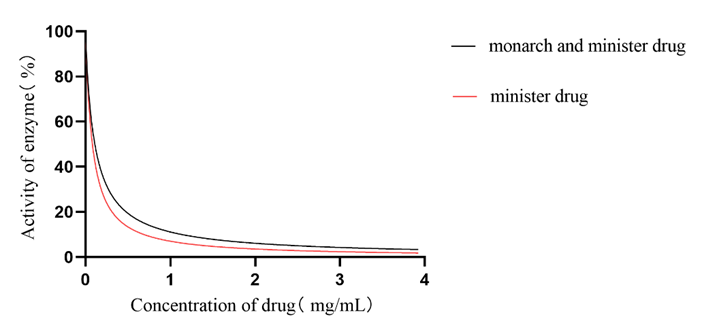
**5.4** **Mode of action of the inhibitory effect of raw herbs from each combination of split formulae in Drug Combination-6s on SARS-CoV-2 3CLpro**

The calculation procedure was as follows: (1) The enzymatic activity efficiency of SARS-CoV-2 3CLpro of each split-side combination of extracts of Drug Combination-6 was deduced from the enzymatic activity efficiency of SARS-CoV-2 3CLpro of raw herbs of each split-side combination of Drug Combination-6 at different concentrations. The concentration of raw herbs of each split-formula combination = concentration of extracts of each split-formula combination × dosage of each split-formula combination/mass of extracts of each split-formula combination. (2) The concentration of raw herbs and the corresponding enzymatic activity efficiency of SARS-CoV-2 3CLpro of each dismantled combination of Drug Combination-6 were imported into GraphPad Prism for graphing, and the Inhibitor vs. normalized response-Variable slope formula was selected to fit the inhibition curve, IC50 and HillSlope. (3) Based on the mathematical model of the "one-band-one-line method", we analyzed the interaction mode (additive, antagonistic or synergistic) between drugs after the addition of different drugs. Taking monarch drugs and monarch-minister drugs as an example, assuming that the mode of action of monarch drugs and minister drugs is additive (i.e., the actual efficacy is equal to the expected additive effect), the addition of minister drugs, the total concentration of monarch-minister drugs raw herbs should be substituted into the equation of the fitted curve of monarch drugs raw herbs. The fitted curve of monarch-minister drugs herbs with monarch drugs as the reference. The fitted equation is

where X represents the concentration of monarch-minister drugs raw material, and Y represents the predicted enzymatic activity efficiency of SARS-CoV-2 3CLpro at different concentrations of monarch-minister drugs raw material. The fitted results of IC50 and HillSlope are as follows:

1. If the fitted curves overlap with the actual monarch-minister drugs fitted curves, it means that the monarch drugs and minister drugs are additive;
2. If the fitted curve is below the actual monarch-minister drugs fitted curve, it means that monarch drugs and minister drugs are antagonistic;
3. If the fitted curve is above the actual monarch-minister drugs fitted curve, it means that monarch drugs and minister drugs are synergistic.

**Analysis of results**



**Fig. 5**

The equation of the fitted curve for the SARS-CoV-2 3CLpro of the raw herb of Junmai in the Drug Combination-6 was obtained by fitting as

(1)

The different concentrations of the monarch and minister crude drugs are brought into the equation (1). The enzyme activity curve of SARS-CoV-2 3CLpro concerning monarch drugs was fitted under the action mode of additive action of monarch drugs and minister drugs. The result is shown in Fig. 5. The fitting curve of the monarch-minister drugs to SARS-CoV-2 3CLpro is below the fitting curve of the real monarch-minister drugs to SARS-CoV-2 3CLpro, which shows that monarch-minister drugs and minister drugs have an antagonistic effect.



**Fig. 6**

The different concentrations of the drugs materials of the monarch and his minister were brought into the equation (1). The enzyme activity curve of SARS-CoV-2 3CLpro with monarch drugs as reference was fitted under the action mode of additive action of monarch drugs and minister assistant. The result is shown in Fig. 6. The fitting curve of the monarch-minister-assistant drugs to SARS-CoV-2 3CLpro is below the fitting curve of the actual monarch-minister-assistant drugs to SARS-CoV-23CLpro, which shows that monarch drugs and minister-assistant drugs are antagonistic. The equation of the fitted curve for SARS-CoV-2 3CLpro of the raw herbs of the monarch-minister drugs in the Drug Combination-6 was obtained by fitting

The different concentrations of the raw herbs of the monarch-minister were brought into the equation (2). The enzyme activity curves of SARS-CoV-2 3CLPro were fitted under the combined action mode of monarch-minister and assistant drugs. The results are shown in Fig. 6, and the fitted curves of the monarch-minister-assistant drugs to SARS-CoV-2 3CLpro with the reference of monarch-minister are below the fitted curves of the actual monarch-minister-assistant drugs to SARS-CoV-2 3CLpro, indicating that the monarch-minister drugs and assistant drugs are antagonistic.



**Fig. 7**

The different concentrations of the drug materials of the monarch-minister- assistant-conductant drugs were brought into the equation (1). The enzyme activity curve of SARS-CoV-2 3CLpro with the reference of monarch drugs was fitted under the action mode of additive action of monarch drugs and minister-assistant-conductant drugs pair. Results as shown in Fig. 7. The fitting curve of the pair of the monarch-minister-assistant-conductant to SARS-CoV-2 3CLpro concerning monarch drugs is below the fitting curve of actual the pair of the monarch-minister-assistant-conductant to SARS-CoV-2 3CLpro, indicating that monarch drugs and minister-assistant-conductant drugs are antagonistic. The different concentrations of the monarch, minister, assistant, and conductant crude drugs were brought into the equation (2). The enzyme activity curve of SARS-CoV-2 3CLpro was fitted according to the combination of monarch-minister drugs and assistant-conductant drugs. The fitting curve of the pair of the monarch-minister-assistant-conductant drugs to SARS-CoV-23CLpro was below the fitting curve of actual the pair of the monarch-minister-assistant-conductant drugs to SARS-CoV-23CLpro, which indicated that monarch-minister drugs and the assistant-conductant drugs had an antagonistic effect. Through fitting, it is concluded that the fitting curve equation of monarch-minister-assistant in Drug Combination-6 to SARS-CoV-23CLpro is

The different concentrations of the drugs materials of the monarch-minister- assistant-conductant drugs were brought into the equation (3)， fitting the SARS-CoV-2 3CLpro enzyme activity curve of monarch-minister-assistant drugs and conductant drugs under the action mode of additive action with monarch-minister-assistant as reference. The fitting curve of monarch-minister-assistant-conductant to SARS-CoV-2 3CLpro is below the fitting curve of actual the pair of the monarch-minister-assistant-conductant to SARS-CoV-23CLpro, indicating that monarch-minister-assistant drugs and conductant drugs are antagonistic.

**6 Inhibitory effects of different combinations of Drug Combination-6 on SARS-CoV-2 PLP**

**6.1 Preparation of extract**

The extract is prepared as described in 5.1.

**6.2 Determination of** **IC50 of SARS-COV 2 PLP by the extract of each disassembled formula of Drug Combination-6.**

Extracts of different combinations of Lianhua-Qingwen capsules were diluted twice with DMSO, with a total of 11 extract concentrations. Prokaryotic expressed and purified by nickel column SARS-CoV-2 PLP and the fluorescent substrate Dabcyl-Z-RLRGG-AMC (synthesized by Nanjing Peptide Industry) solubilized with Mili-Q were diluted with enzyme activation buffer (20 mM Tris-buffer (pH 8.0), 4 mM DTT). SARS-CoV-2 PLP 40uL (final concentration of 100nM) was incubated with 10uL of extracts of each dismantled combination of Drug Combination-6 at different concentrations for 30min at room temperature, and then the fluorescent substrate (final concentration of 30μM) was added for immediate fluorescence intensity detection. The assay conditions are as follows. Excitation: 336/20, emission: 490/20, interval 30s, shaking plate 0.01s for 1h, and gain value 100. To exclude the effect of different concentrations of extracts of each dismantled combination of Drug Combination-6 on the fluorescence intensity, a control without SARS-CoV-2 PLP was set.

The calculation method is as follows. Enzyme activity efficiency = [(fluorescence signal at the endpoint of detection of each combination of extracts of different concentrations of Drug Combination-6 - fluorescence signal at the beginning of detection) - (fluorescence signal at the endpoint of detection of each combination of extracts of different concentrations of Drug Combination-6 without SARS-CoV-2 PLP under the same conditions - fluorescence signal at the beginning of detection)]/[( fluorescence signal at the detection endpoint of positive control DMSO - fluorescence signal at the detection starting point) – (no SARS-CoV-2 PLP DMSO detection endpoint fluorescence signal under the same conditions - detection starting point fluorescence signal)] ×100. The concentrations and the corresponding enzymatic activity efficiencies of SARS-CoV-2 PLP for each extract of the dismantled combination of Drug Combination-6 were imported into GraphPad Prism. The IC50 of the drugs was fitted by choosing Inhibitor vs. normalized response-Variable slope.

**Experimental results**



**Fig. 8**

As shown in Fig. 8, with the increase of the concentration of monarch drugs extract, the activity of SARS-CoV-2 PLP decreased gradually, indicating that monarch drugs extract had an inhibitory effect on SARS-CoV-2 PLP, and its IC50 was 0.1495 mg/ml.



**Fig. 9**

As shown in Fig. 9, with the increase of the concentration of monarch-minister drugs extract, the activity of SARS-CoV-2 PLP decreased gradually, indicating that the monarch-minister drugs extract had an inhibitory effect on SARS-CoV-2 PLP, and its IC50 was 0.0858 mg/ml.



**Fig. 10**

As shown in Fig. 10, with the increase of the concentration of monarch-minister- assistant extract, the activity of SARS-CoV-2 PLP decreased gradually, indicating that monarch- minister-assistant drugs extract had an inhibitory effect on SARS-CoV-2 PLP, and its IC50 was 0.0616 mg/ml.



**Fig. 11**

As shown in Fig. 11, with the increase of the concentration of the monarch-minister-assistant-conductant drugs extract, the activity of SARS-CoV-2 PLP decreased gradually, indicating that the monarch-minister-assistant-conductant drugs extract had an inhibitory effect on SARS-CoV-2 PLP, and its IC50 was 0.1649 mg/ml.

**6.3 Calculation of IC50 of SARS-CoV-2 PLP by the original drugs materials of the disassembled combinations of Drug Combination-6**

According to the IC50 of the extract of Drug Combination-6 to SARS-CoV-2 PLP, the IC50 of the crude drugs of Drug Combination-6 to SARS-CoV-2 PLP can be calculated. The calculated formula is each disassembled prescription of Drug Combination-6 to the IC50 of SARS-CoV-2 PLP × the dosage of the extract of each disassembled prescription / the quality of the extract of each combination of the disassembled prescription.

**Table 5. IC50 of each disassembled prescription of Drug Combination-6**

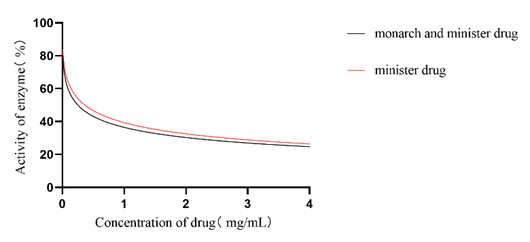
|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Disassembly combination | Dosage (gram) | | IC50 of Crude drug materials (mg/mL) | Extract quality  (gram) | IC50 of extract (mg/mL) |
| Monarch | | 10.2 | 0.3657 | 4.17 | 0.1495 |
| Monarch-minister | | 18.7 | 0.2527 | 6.35 | 0.0858 |
| Monarch-minister-assistant | | 38.57 | 0.1846 | 12.87 | 0.0616 |
| Monarch-minister-assistant- conductant | | 40.27 | 0.4514 | 14.71 | 0.1649 |

As shown in Table 5, the IC50 of monarch drugs, monarch-minister drugs, monarch-minister-assistant drugs and monarch-minister-assistant-conductant drugs to SARS-CoV-2 PLP are 0.3657 mg/mL, 0.2527 mg/mL, 0.1846 mg/ml and 0.4514 mg/mL, respectively. Therefore, the best inhibitory effect on SARS-CoV-2 PLP is a monarch-minister-assistant drugs, followed by monarch-minister drugs, monarch drugs, and monarch-minister-assistant-conductant drugs.

**6.4 The inhibitory effect of different combinations of crude drugs in Drug Combination-6 on SARS-CoV-2 PLP**

The calculation process is described in 6.4, except that the proteins used are different.

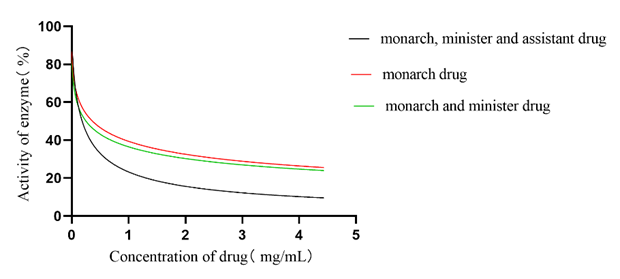
**Result analysis**

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**Fig. 12**

Through fitting, it is concluded that the fitting curve equation of monarch crude drugs in Drug Combination-6 to SARS-CoV-2 PLP is

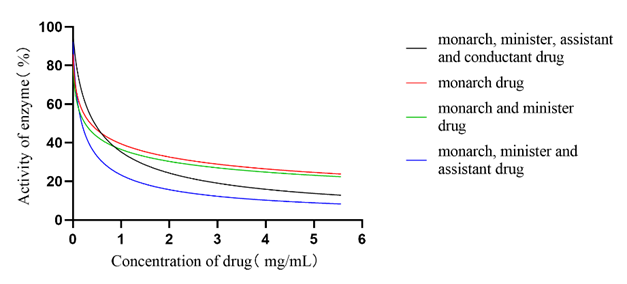
The different concentrations of the monarch and minister crude drugs were brought into the equation (4). The enzyme activity curve of SARS-CoV-2 PLP concerning monarch drugs was fitted. Results as shown in Fig. 12. The fitting curve of the monarch-minister drug to SARS-CoV-2 PLP with the reference of monarch drugs is above the fitting curve of the actual monarch-minister drugs to SARS-CoV-2 PLP, indicating that monarch drugs and minister drugs have a synergistic effect.

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**Fig. 13**

The different concentrations of the drugs materials of the monarch-minister-assistant of the crude drugs were brought into the equation (4). The enzyme activity curve of SARS-CoV-2 PLP with monarch drugs as reference was fitted under the action mode of additive action of the monarch-minister-assistant drugs. Results as shown in Fig. 13. The fitting curve of monarch-minister-assistant to SARS-CoV-2 PLP is above the fitting curve of monarch-minister-assistant to SARS-CoV-2 PLP, which shows that monarch drugs and minister-assistant have a synergistic effect. The equation of the fitted curve for the SARS-CoV-2 PLP of the raw herbs of the monarch-minister crude drugs in the Drug Combination-6 was obtained by fitting it as

The different concentrations of the drugs materials of the monarch-minister-assistant were brought into the equation (5). The enzyme activity curve of SARS-CoV-2 PLP under the action of monarch-minister-assistant was fitted with the reference of the monarch-minister drugs. The fitting curve of monarch-minister-assistant to SARS-CoV-2 PLP is above the fitting curve of actual monarch-minister-assistant to SARS-CoV-2 PLP, indicating that monarch-minister drugs and assistant have a synergistic effect.



**Fig. 14**

The different concentrations of the drugs materials of the monarch-minister-assistant-conductant were brought into the equation

The enzyme activity curve of SARS-CoV-2 PLP under the action of the monarch-minister-assistant-conductant drugs was fitted with the monarch drugs as a reference. Results as shown in Fig. 14. There is an intersection point between the fitting curve of the monarch-minister-assistant-conductant drugs to SARS-CoV-2 PLP about monarch drugs and the fitting curve of the actual monarch-minister-assistant-conductant drugs to SARS-CoV-2 PLP. When the concentration of a monarch-minister-assistant-conductant crude drugs is less than the concentration of intersection point, the fitting curve of monarch-minister-assistant-conductant to SARS-CoV-2 PLP is at the bottom of the fitting curve of SARS-CoV-2 PLP with a monarch as reference. It shows that monarch drugs and minister-assistant-conductant drugs are antagonistic. When the concentration of a monarch-minister-assistant-conductant crude drugs is higher than the concentration of intersection point, the fitting curve of monarch-minister-assistant-conductant to SARS-CoV-2 PLP is above the fitting curve of monarch-minister-assistant-conductant to SARS-CoV-2 PLP with a monarch as a reference, which shows that monarch and minister-assistant-conductant crude drugs have a synergistic effect. The different concentrations of the drugs materials of the monarch-minister and assistant-conductant crude drugs were brought into the equation (6), it the SARS-CoV-2 PLP enzyme activity curve of monarch-minister drugs and assistant-conductant drugs with the monarch-minister drugs as reference under the mode of additive action. Results as shown in Fig. 14. There is an intersection point between the fitting curve of the monarch-minister-assistant-conductant drugs to SARS-CoV-2 PLP regarding monarch-minister drugs and the fitting curve of the actual monarch-minister-assistant-conductant drugs to SARS-CoV-2 PLP. When the concentration of a monarch-minister-assistant-conductant crude drugs is less than the concentration of intersection point, the fitting curve of monarch-minister-assistant-conductant to SARS-CoV-2 PLP is at the bottom of the fitting curve of SARS-CoV-2 PLP with monarch-minister as reference. It shows that monarch-minister and assistant-conductant drugs are antagonistic. When the concentration of a monarch-minister-assistant-conductant crude drugs is higher than the concentration of intersection point, the fitting curve of monarch-minister-assistant-conductant to SARS-CoV-2 PLP is above the fitting curve of monarch-minister-assistant-conductant to SARS-CoV-2 PLP with monarch-minister as a reference, which shows that monarch-minister and assistant-conductant have a synergistic effect. Through fitting, it is concluded that the fitting curve equation of monarch-minister-assistant in Drug Combination-6 to SARS-CoV-2 PLP is

The different concentrations of the drugs materials of the monarch-minister-assistant-conductant were brought into the equation (7). The enzyme activity curve of SARS-CoV-2 PLP under the action of monarch-minister-assistant and the conductant drugs was fitted according to the reference of monarch-minister-assistant. The fitting curve of monarch-minister-assistant-conductant to SARS-CoV-2 PLP is below the fitting curve of monarch-minister-assistant-conductant to SARS-CoV-2 PLP with monarch-minister-assistant as a reference, indicating that monarch-minister-assistant and conductant drugs are antagonistic.