

# Antibacterial Activity Of Shin'iseihaito (Xinyiqingfeitang) And its Components Against Methicillin-Resistant *Staphylococcus aureus*

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## History

- Submission Date: 11-01-17;
- Review completed: 02-03-17;
- Accepted Date: 13-03-17

DOI : 10.5530/pj.2017.3.53

## Article Available online

<http://www.phcogj.com/v9/i3>

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## ABSTRACT

**Objective:** Shin'iseihaito (xinyiqingfeitang in Chinese, SSHT), a formula in traditional Japanese Kampo medicine and Chinese medicine comprising nine crude drugs, Gypsum, Ophiopogon Tuber, Scutellaria Root (SR, root of *Scutellaria baicalensis*), Gardenia Fruit, Anemarrhena Rhizome, Lilium Bulb, Magnolia Flower, Loquat Leaf, and Cimicifuga Rhizome (CR, rhizome of *Cimicifuga heracleifolia*), is commonly used to treat sinusitis associated with purulent nasal discharge and reddish nasal mucosa. We evaluated anti-bacterial activity of SSHT extract on methicillin-resistant *Staphylococcus aureus* (MRSA), one cause of bacterial sinusitis. **Materials and Methods:** Sterile paper disks impregnated with SSHT extract, the combination of crude drugs composing SSHT according to the traditional pharmacological theory, or each component were placed on Mueller-Hinton agar plates inoculated with several strains of MRSA isolated from the patients. The diameter of inhibitory zone was measured after 18–24 h incubation. **Results:** SSHT extract showed antibacterial activity against 128/190 (66.8%) MRSA clinical isolates. The effect of the extract of SSHT without *heat-clearing* drugs (SSHT–HC) or without *exterior-releasing* drugs (SSHT–ER) were significantly lower than that of SSHT extract. Each water extract of SR, Loquat Leaf, Magnolia Flower and CR showed significant anti-MRSA activity, and SR extract exhibited the largest inhibitory zone. **Conclusions:** SSHT has antibacterial activity against MRSA clinical isolates, and SR mainly contributes to the antibacterial activity of SSHT against MRSA clinical isolates.

**Key words:** Shin'iseihaito, Sinusitis, Methicillin-resistant *Staphylococcus aureus* (MRSA), Antibacterial activity, *Scutellaria baicalensis*.

## INTRODUCTION

Sinusitis is the commonest disease, and decreases quality-of-life as a result of symptoms such as nasal discharge, nasal congestion, hyposmia, facial pain or pressure, headache, and malaise.<sup>1–3</sup> Bacterial sinusitis can cause rare but severe orbital and intracranial complications such as preseptal or orbital cellulitis, subperiosteal or orbital abscess, cavernous sinus thrombosis, subdural or epidural empyema, cerebritis, brain abscess and meningitis.<sup>4</sup>

One of pathogens for bacterial sinusitis is methicillin-resistant *Staphylococcus aureus* (MRSA),<sup>5,6</sup> and recently, the rate of recovery of MRSA is increasing in patients with sinusitis.<sup>7</sup> MRSA is resistant to  $\beta$ -lactams, and often resistant to other antibacterial agent such as macrolides, tetracyclines, aminoglycosides, and fluoroquinolones.<sup>8</sup> Vancomycin is prescribed for the treatment of MRSA infections,<sup>8</sup> however, the emergence of vancomycin-resistant *Staphylococcus aureus* was reported.<sup>9,10</sup> Therefore, the treatment other than antibiotic therapy is demanded.

Shin'iseihaito (xinyiqingfeitang in Chinese, SSHT) is a formula in traditional Japanese Kampo medicine and Chinese medicine to treat sinusitis associated with *lung-heat* syndrome, which is characterized by the symptoms of purulent nasal discharge, reddish

nasal mucosa, and so on.<sup>11</sup> SSHT composes nine crude drugs, Gypsum (G), Scutellaria Root (SR), Gardenia Fruit (GF), Anemarrhena Rhizome (AR), Ophiopogon Tuber (OT), Lilium Bulb (LB), Loquat Leaf (LL), Magnolia Flower (MF), and Cimicifuga Rhizome (CR).<sup>11</sup> In our previous study, we showed the anti-infectious effect of SSHT extract against pneumococcus-infected mice,<sup>12</sup> and the antibacterial activity of SSHT extract against *Streptococcus pneumoniae in vitro*.<sup>13</sup> However, the antibacterial activity of SSHT against MRSA has not been investigated.

In this study, we evaluated the antibacterial activities of SSHT hot water extract and its constituents against MRSA *in vitro*, and discussed the contribution of each crude drugs composing SSHT according to the traditional medicinal theory.

## MATERIALS AND METHODS

### Preparation of SSHT

The materials of SSHT were the same lots of crude drugs in our previous study.<sup>13</sup> Each crude drug (5 g), the mixture of crude drugs constituting SSHT (total 28 g, Table 1) or each SSHT prescription (Table 2) were boiled in 20-times weight of distilled water

**Cite this article:** Konishi T, Minami M, Makino T. Antibacterial Activity Of Shin'iseihaito (Xinyiqingfeitang) And its Components Against Methicillin-Resistant *Staphylococcus aureus*. Pharmacogn J. 2017;9(3):310-4.

for 30 min and filtrated, lyophilized, and the dried powdered extracts of SSHT, each crude drug or each SSHT prescription were weighed. The yields of the extracts to the original crude drugs were shown in Table 1 and Table 2. The powdered extracts were suspended in distilled water and stored in  $-20^{\circ}\text{C}$  until use. The fingerprint chromatogram of SSHT extract was almost similar to the previous study.<sup>13</sup>

#### Bacterial strains and disk diffusion method

One hundred ninety methicillin-resistant *Staphylococcus aureus* (MRSA) clinical isolates were obtained from 190 different patients admitted to Daido Hospitals, Nagoya, Japan. All *Staphylococcus* isolates were identified by standard conventional biochemical methods or the VITEK2 system (bioMérieux, Durham, NC, USA). Minimal inhibitory concentrations (MICs) were determined at clinical laboratory in Daido Hospital using broth micro dilution methodology with the VITEK2 system. Evaluation of susceptibilities were calculated based on Clinical Laboratory Standard Institute (CLSI) break point. Antibacterial activity was evaluated by the disk diffusion method, as described previously.<sup>13</sup> Briefly, sterile paper disks (12 mm) were impregnated with samples, and dried up at room temperature. The colonies of MRSA which cultured overnight on LB agar (Sigma-Aldrich, St. Louis, MO, USA) at  $37^{\circ}\text{C}$ , were collected into sterile saline and adjusted to 0.5 McFarland using sterile saline, and inoculated on Mueller-Hinton agar (Becton Dickinson, Franklin Lakes, NJ, USA). The paper disks were placed on the plates, and were incubated at  $37^{\circ}\text{C}$  for 18–24 h. Then, the diameter of inhibitory zone was measured.

#### Statistical Analysis

All statistical analyses were performed using the PASW Statistics version 18 (SPSS, IBM, Armonk, NY, USA). The statistical analysis was conducted using a one-way analysis of variance (ANOVA) followed by Bonferroni/Dunnnett's multiple *t*-test for the differences among multiple groups. *P*-values less than 0.05 were considered statistically significant.

## RESULTS AND DISCUSSION

Antibacterial activity screening test of SSHT extract against 190 MRSA clinical isolates was evaluated by using disk diffusion method. SSHT extract (7.5 mg/disk) exhibited antibacterial activity against 127 MRSA clinical isolates (66.8%). Among them, we selected 3 MRSA clinical

isolates (A, DDM147; B, DDM179; C, DDM198) from which SSHT extract exhibited the strongest anti-bacterial effects, and investigated the contribution of each crude drug composing of SSHT. SSHT composes G, SR, GF and AR as *heat-clearing* drugs to treat internal *heat*-syndrome, MF and CR as *exterior-releasing* drugs to treat the *exterior* syndromes of *wind-cold* and *wind-heat*, and others.<sup>14</sup> In Kampo medicine, yellowish discharge from nasal cavities which relates to the bacterial infection is the reflection of *lung-heat* and the infection of *exterior wind-heat* pathogen. Then, we considered these drug groups might contribute the anti-MRSA effects of SSHT. We prepared the extracts of SSHT without *heat-clearing* drugs (SSHT-HC) or *exterior-releasing* drugs (SSHT-ER) to evaluate the contribution of these groups to the effect of SSHT. As shown in Figure 1, the antibacterial activities of SSHT-HC (3.0 mg/disk) and SSHT-ER extract (6.5 mg/disk) against 3 MRSA clinical isolates were significantly decreased compared with SSHT extract (7.5 mg/disk), and the antibacterial activity of SSHT-HC extract was decreased to control level. The results suggest that *heat-clearing* drugs mainly contribute the anti-MRSA activity of SSHT, and *exterior-releasing* drugs partially contribute it.

We investigated anti-MRSA activity of the extract of SSHT without each crude drug consisting of SSHT. As shown in Figure 2, Anti-MRSA activities of the extract of SSHT without G, GF, AR, OT, LB, or MF were equal compared with SSHT extract, however, those of the extract of SSHT without LL were significantly decreased in 1 strain, those of the extract of SSHT without CR were significantly decreased in 2 strains, and those of the extract of SSHT without SR were significantly decreased in 3 strains compared with SSHT extract, and the anti-MRSA activity of SSHT without SR was disappeared to the control level. These results suggest that SR mainly, and LL and CR slightly, contributes anti-MRSA activity of SSHT against MRSA. The diameter of inhibitory zone of SSHT-CR extract in 3 MRSA clinical isolates were decreased 0.6 – 1.0 mm and SSHT-MF extract in them were equal compared with SSHT extract. In contrast, the diameter of inhibitory zone of SSHT-ER extract in them were decreased 1.3 – 2.0 mm. It is considered that SR is the representatives of *heat-clearing* drugs and the presence of a synergistic or additional effects between CR and MF in *exterior-releasing* drugs in SSHT.

We screened the antibacterial activity of the hot water extracts of each crude drug composing SSHT. As shown in Figure 3, hot water extracts

**Table 1: Compositions of shin'iseihaito (SSHT).**

Name of crude drug <sup>a</sup>	Weight <sup>b</sup> (g)	Ratio of yield <sup>c</sup> (%)
Gypsum (G)	6.0	1.6
Scutellaria Root (SR)	3.0	42
Gardenia Fruit (GF)	1.5	32
Anemarrhena Rhizome (AR)	3.0	37
Loquat Leaf (LL)	1.0	19
Ophiopogon Tuber (OT)	6.0	36
Lilium Bulb (LB)	3.0	20
Magnolia Flower (MF)	3.0	14
Cimicifuga Rhizome (CR)	1.5	18
Shin'iseihaito (SSHT)	28	23

<sup>a</sup>All crude drugs are registered in Japanese Pharmacopoeia 17th Edition<sup>19</sup>

<sup>b</sup>Weight of each herbal medicine in a dairy dosage of SSHT. This formula is described in the guideline of over-the-counter Kampo medicine published from Ministry of Health, Labour and Welfare, Japan, 2012.

<sup>c</sup>The decoctions were prepared according to experimental design, and ratio of yield means % of dried weight of the decoction to the original crude drug.

**Table 2: Weight and ratio of yield of each modified SSHT prescription.**

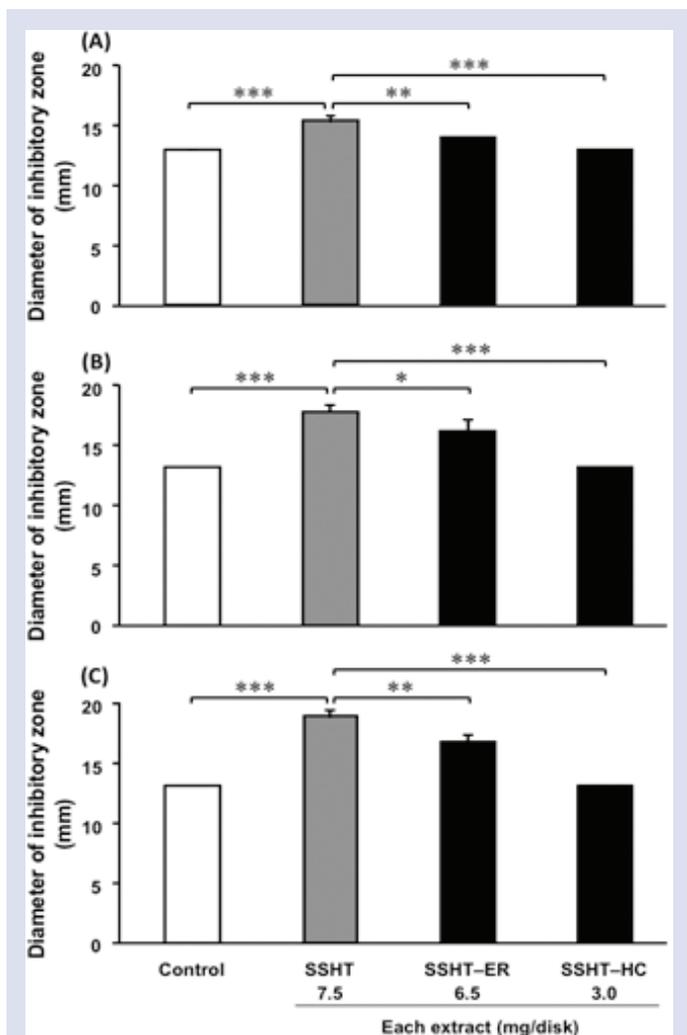
Modified SSHT	Weight <sup>a</sup> (g)	Ratio of yield <sup>b</sup> (%)
SSHT without <sup>d</sup> <i>heat-clearing</i> drugs (SSHT-HC)	14.5	18
SSHT without <sup>e</sup> <i>exterior-releasing</i> drugs (SSHT-ER)	23.5	24
SSHT without G (SSHT-G)	22.0	25
SSHT without SR (SSHT-SR)	25.0	19
SSHT without GF (SSHT-GF)	26.5	21
SSHT without AR (SSHT-AR)	25.0	22
SSHT without LL (SSHT-LL)	27.0	20
SSHT without OT (SSHT-OT)	22.0	22
SSHT without LB (SSHT-LB)	25.0	21
SSHT without MF (SSHT-MF)	25.0	23
SSHT without CR (SSHT-CR)	26.5	23

<sup>a</sup>Weight of each herbal medicine related to the dairy dosage of SSHT.

<sup>b</sup>The decoctions were prepared according to experimental design, and ratio of yield means % of dried weight of the decoction to the original crude drug.

<sup>c</sup>Heat-clearing drugs refers to G, SR, GF and AR.

<sup>d</sup>Exterior-releasing drug refers to MF and CR.

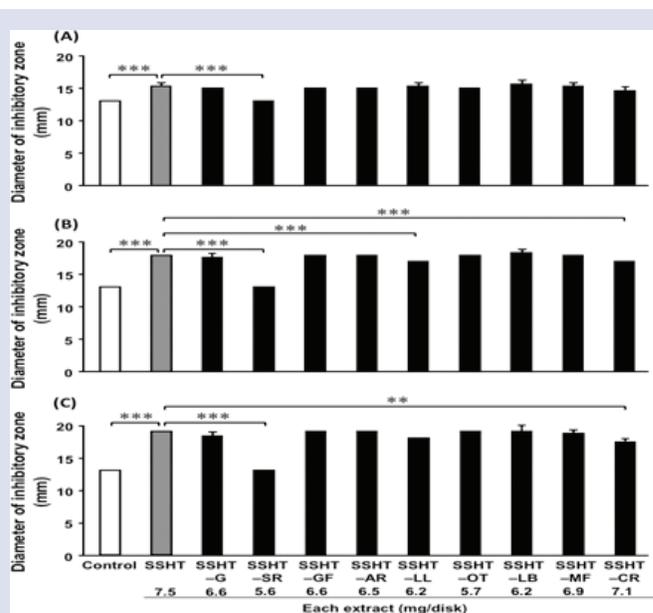


**Figure 1 :** Antibacterial effect of the extract of SSHT, SSHT-HC and SSHT-ER against 3 MRSA clinical isolates. Paper disks containing SSHT, SSHT-HC, or SSHT-ER extract were put on placed on the plates inoculated with each MRSA clinical isolate (A, DDM147; B, DDM179; C, DDM198), and the diameter of inhibitory zone after 18 – 24 h incubation was measured. The amount of SSHT-HC and SSHT-ER was equivalent to SSHT extract (7.5 mg/disk). Values are expressed as the mean  $\pm$  S.D. ( $n = 3$ ). \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  evaluated by Bonferroni/Dunnet's multiple  $t$ -test.

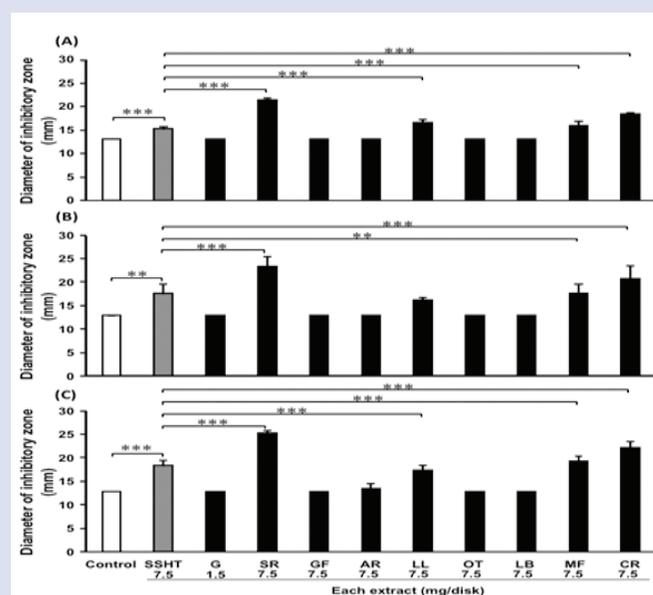
of SR, CR, MF and LL showed significant anti-MRSA clinical isolates, and hot water extracts of SR exhibited the strongest antibacterial activity among 3 strains.

In the theory of traditional Chinese medicine described in the textbook,<sup>14</sup> SR clears *heat* and dries *dampness*, resolves toxicity in the upper burner, and calms the fetus, LL transforms *phlegm* and clears *lung's heat*, CR discharges exterior condition, and resolves toxicity in the upper, and MF expels *wind-cold* and unblocks the nasal passages. As regards other crude drugs composing SSHT, OT, GF, AR, and G also clear *heat* in the traditional theory.<sup>14</sup> Therefore, we consider that the term *heat* in traditional theory and bacterial infection are not equal because not all crude drugs that have the effectiveness of clearing *heat* exhibit antibacterial activity.

In the present study, we showed the hot water extract of SSHT had the antibacterial activity against MRSA. We previously reported that the hot water extract of SR had the antibacterial activity against *Streptococcus*



**Figure 2 :** Antibacterial effects of the extract of SSHT without single crude drug consisting of SSHT against 3 MRSA clinical isolates. Paper disks containing SSHT extract or SSHT-G, SSHT-SR, SSHT-GF, SSHT-AR, SSHT-LL, SSHT-OT, SSHT-LB, SSHT-MF and SSHT-CR were put on placed on the plates inoculated with each MRSA clinical isolate (A, DDM147; B, DDM179; C, DDM198), and the diameter of inhibitory zone after 18 – 24 h incubation was measured. Values are expressed as the mean  $\pm$  S.D. ( $n = 3$ ). \*\* $P < 0.01$ , \*\*\* $P < 0.001$  evaluated by Bonferroni/Dunnet's multiple  $t$ -test.



**Figure 3 :** Antibacterial effects of SSHT extract and the extracts of 9 crude drugs consisting of SSHT against 3 MRSA clinical isolates. Paper disks containing SSHT extract or each extract of G, SR, GF, AR, LL, OT, LB, MF and C were put on placed on the plates inoculated with each MRSA clinical isolate (A, DDM147; B, DDM179; C, DDM198), and the diameter of inhibitory zone after 18 – 24 h incubation was measured. Values are expressed as the mean  $\pm$  S.D. ( $n = 3$ ). \*\* $P < 0.01$ , \*\*\* $P < 0.001$  evaluated by Bonferroni/Dunnet's multiple  $t$ -test.

*pneumoniae*.<sup>13</sup> In addition, it is reported that the hot water extract of SR had the antibacterial activity against *Propionibacterium acnes*<sup>15</sup> and *Helicobacter pylori*,<sup>16</sup> and 60% ethanol extract of SR had antibacterial activity against *Staphylococcus aureus*,<sup>17</sup> *Listeria monocytogenes*, and *Salmonella enterica* serovars Kentucky, Senftenberg, Enteritidis and Typhimurium.<sup>18</sup> *P. acnes* and *Staphylococcus aureus* belong to gram-positive bacteria, but *H. pylori* and *Salmonella enterica* are gram-negative. It is suggested that SR water extract would have broad spectrum antibacterial activity. Bacterial sinusitis is frequently caused by *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Streptococcus pyogenes* other than MRSA. These bacteria are also gram-positive or gram-negative bacteria. Therefore, SSHT may exhibit antimicrobial activity in these causative bacteria of sinusitis.

We previously measured the amount of baicalin in SR extract using HPLC because baicalin is the marker compounds of SR<sup>13</sup>, and the content of baicalin in SR extract was 33 (w/w) %. This means 7.5 mg of SR extract contains 2.5 mg of baicalin. Baicalin has the antibacterial activities against *Streptococcus pneumoniae*,<sup>13</sup> *Sarcina lutea*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella enterica* serovars Kentucky, Senftenberg, Enteritidis and Typhimurium.<sup>18</sup> It is considered that baicalin may active component of SSHT extract against MRSA.

## CONCLUSION

The hot water extract of SSHT, a formula in traditional Japanese Kampo medicine and Chinese medicine, showed the antibacterial activity against MRSA, and among the components of SSHT, SR mainly contributes antibacterial activity of SSHT against MRSA. Although SSHT contains other crude drugs than SR that have anti-MRSA activity and these crude drugs might exhibit synergistic activity, this effect of crude drugs might be related to one of the action mechanisms of SSHT to treat bacterial sinusitis.

## ACKNOWLEDGEMENT

Nil

## CONFLICT OF INTEREST

None

## ABBREVIATIONUSED

**ANOVA:** A one-way analysis of variance; **AR:** Anemarrhena Rhizome; **CR:** Cimicifuga Rhizome; **GF:** Gardenia Fruit; **G:** Gypsum; **LB:** Lilium Bulb; **LL:** Loquat Leaf; **MF:** Magnolia Flower; **OT:** Ophiopogon Tuber; **SR:** Scutellaria Root; **SSHT:** Shin'iseihaito (xinyiqingfeitang in Chinese).

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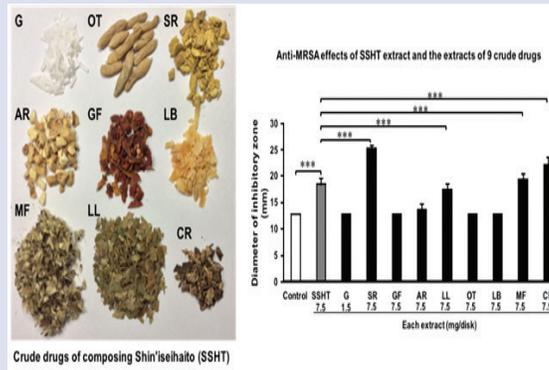
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## SUMMARY

- Shin'iseihaito (xin yi qing fei tang in Chinese, SSHT), a formula in traditional Japanese Kampo medicine and Chinese medicine comprising nine crude drugs, showed antibacterial effect against methicillin-resistant *Staphylococcus aureus* (MRSA).
- Each water extract of *Scutellaria* Root, Loquat Leaf, *Magnolia* Flower and *Cimicifuga Rhizome* showed significant anti-MRSA activity, and *Scutellaria* Root extract exhibited the largest inhibitory zone.

GRAPHICAL ABSTRACT



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**Cite this article:** Konishi T, Minami M, Makino T. Antibacterial Activity Of Shin'iseihaito (Xinyiqingfeitang) And its Components Against Methicillin-Resistant *Staphylococcus aureus*. Pharmacogn J. 2017;9(3):310-4.