



## Modified transfusion devices, inducer, and procedure for agarwood-inducing by infusion technique

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### ABSTRACT

“The Infusion Technique” is a relatively effective method to induce agarwood formation in *Aquilaria sinensis* trees, while there are many defects in the existing transfusion devices, inducers and procedures. Our study evaluated a novel Modified Kit for “the Infusion Technique”, which is developed on five Chinese patents, including the plant transfusion needle and vessel, inducer and procedure (for primary and secondary whole tree agarwood-inducing process). The plant transfusion needle prevent the transfusion fluid from oozing out of the trunks, thus improve infusion efficiency. The transfusion vessel make the clogging in transfusion needles or tubes easily detected, then quickly handled. By adopting Modified Kit, the agarwood was formed in the whole *Aquilaria* trunks at the three-month after treatment, the average ASEC (Alcohol Soluble Extractive Content) in all agarwood obtained at the six-month met Chinese Pharmacopoeia standard, and reached the peak value of 43.9% at the 16-month. These results indicate that the Modified Kit contributes to improve the quality of agarwood and shorten the Production Period.

**Keywords:** Agarwood-inducing, *Aquilaria sinensis*; Inducer; Modified Kit; Primary and secondary agarwood-inducing procedure; Transfusion needle; Transfusion vessel.

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### INTRODUCTION

*Aquilaria sinensis*, an *Aquilaria* spp., within the thymelaeaceae family, is the only source in China to induce agarwood, one of the valuable aromatic medicinal herbs. Agarwood is not produced in healthy wood of *Aquilaria* Trees, but formed only when wounded by certain natural factors, such as lightning strike, insect attack or microbial invasion, or by artificial processes, such as axe chopping, hole drilling or fungi inoculation. In recent years, the price of agarwood has been continuously rising due to the huge market demand and low productivity. The development of agarwood industry is hitting a bottleneck mainly attributed to a long production period, the low yield by natural-wounding methods, and the barriers to popularize the traditional artificial Agarwood-Inducing Techniques [1-2].

To date, the “Infusion Technique”, that is, to infuse agarwood-inducers composed of phytohormones and certain chemical agents into *A. sinensis* to induce agarwood formation, has been proved to be a relatively effective method [3-5]. However, the defects in the existing transfusion sets, inducers and procedures may lead to damage, rot, even death of large areas of *Aquilaria* barks and trunks.

Based on the above situation, A Modified Kit for the “Infusion Technique”, including the transfusion needle and vessel, inducer and procedure (for primary and secondary agarwood-inducing process) was developed and applied for five Chinese patents for the Modified Kit: three invention patents [6-8] and two utility model patents [9-10].

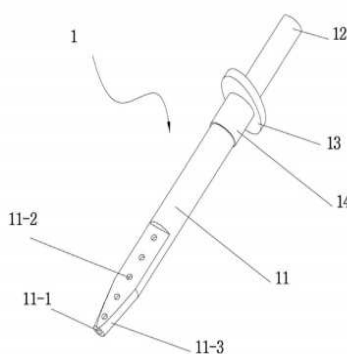
## EXPERIMENTAL SECTION

### Plant materials

*Aquilaria sinensis* Lour. Gilg Trees, identified by prof. Zhi-Jian Feng, South China Agricultural University, China, were obtained from Zhongshan Agarwood Huang Agroforestry Development Co., Ltd for Agarwood-Inducing by Modified Kit.

### The transfusion needle in Modified Kit

Currently, the transfusion needles used in the “Infusion Technique” are either transformed medical injection needles or the traditional plant transfusion needle once acquired a Chinese patent [11]. However, both types of needles carry certain flaws which usually result in a compromised infusion performance. To iron out these flaws, we designed a plant transfusion needle (Figure 1), and Table 1 shows the comparison of the three types of needles.



**Fig. 1 The plant transfusion needle in Modified Kit**

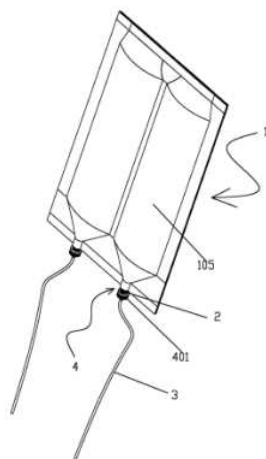
1) needle, 11) needle body, 11-1) fluid outlet , 11-2) drain hole, 11-3) needle tip, 12) connector , 13) projecting shoulder , 14) squeezing part

**Table 1 The comparison of transformed medical injection needles, traditional plant transfusion needle and the plant transfusion needle in Modified Kit**

Needle type	Main features
Transformed medical injection needle	The fluid outlet is generally located in needle tip, and prone to be blocked by bits of xylem wood, resulting in a compromised infusion performance.
Traditional plant transfusion needle	Multiple-fluid-outlets designing partly prevents from being blocked; The fluid outlets are squeezed tightly by wood tissue when the needle being inserted into the trunks, reducing the infusion efficiency.
The plant transfusion needle in Modified Kit	With a sunken-style designing in the front end, a liquid storage cavity is formed between the fluid outlets and hole walls when the needle being inserted into the trunks, which completely prevents the fluid outlets from being blocked, and the fluid from oozing out of the trunks, thus improves the infusion efficiency.

### The transfusion vessel in Modified Kit

Figure 2 shows the design of transfusion vessel in Modified Kit, and Table 2 shows its comparison to traditional infusion vessel.



**Fig. 2 The transfusion vessel in Modified Kit**

(1) vessel body, (105) plastic substrate, (2) vessel spout, (3) infusion tube, (4) connective structure, (401) spout cover

**Table 2 The comparison of traditional infusion vessel and the infusion vessel in Modified Kit**

Vessel type	Main features
Traditional transfusion vessel	<p>The vessel is connected to an transfusion tube which extends to form two branching ducts, each with a needle nailed into either side of the tree trunks.</p> <p>If either side of the needle or branching duct is clogged, the liquid in the vessel will keep on flowing out through the other side and the water level in the vessel will still be lowering gradually. So, the clogging would be hardly detected, leading to a poor infusion on its side.</p>
The transfusion vessel in Modified Kit	<p>The vessel is divided into two or more independent chambers, each connecting to an transfusion tube and needle.</p> <p>If any of the needle or tube is clogged, the water level in the connected chamber will stop lowering, prompting the clog to be detected and handled in time, thus ensuring an effective infusion.</p>

### The inducer in Modified Kit

The constituents were formulated in percent by volume (V/V): 5-10% liquid *Schizophyllum commune*, 1.5-2.0% glucose (sugar), 0.5-1.0% acetic acid (malic acid), 85-95% water; The constituents for culture medium of liquid *Schizophyllum commune* were formulated in mass ratio: 10-120 parts potato, 25-30 parts bran (barley), 20-25 parts glucose, 1000 parts water.

### The procedure for primary whole tree agarwood-inducing

1. Screening and selection of *A. senensis* trees: in situ *Aquilaria* trees with intense sunlight, big canopies, flourishing leaves, undamaged barks, and long plant distance were selected.
2. Pretreatment: moved the light-blockers around the trees, weeded and applied fertilizer until the trees grew strong and stored sufficient nutrients.
3. Formulation of inducer: as elaborated above.
4. Infusion-holes drilling: The trunks within 400 mm above the ground were adopted for drilling holes. The number of the holes was determined based on the Diameter at Breast Height (Generally, two holes in trunks with a DBH less than 100 mm, and one more hole for every 50 mm increase in DBH), and the depth should be considered not to hurt the primary xylem.
5. Infusion: The infusion sets, after being assembled, were hung up at higher trunks, with needles inserted into the infusion-holes. Ensure that the inducer in every chamber of the vessels was continuously infused into the trunks. Then gripped the projecting shoulder and pulled the needle out when infusion was completed.
6. Infusion holes protection: plastic or wax beads were placed into the holes, to help healing of the plant tissues, and to prevent the formed resin from volatilization.
7. Maintenance: applied fertilizers such as livestock or human feces at one-month and six-month, respectively, after drilling holes, and kept insects at bay.
8. Resinous wood harvesting: Before harvest, partially or completed cut off the sieve tubes or pith tissues at one or multiple planes of the trunks or branches. This would help to build up the nutrients in the trunks, and prevent them to be

transported to the roots. When harvesting, reaped the trunks with aromatic resin, cut off the white woods and decayed parts, shade-dried to obtain resinous wood.

#### The procedure for secondary agarwood-inducing

When the Infusion Technique was adopted for agarwood-inducing, the plant tissues, by self-preservation mechanism, usually form a protective layer around the agarwood-inducing area (named as primary agarwood-inducing area). The inducer can not penetrate through the protective layer and stimulate the plant tissues outside the layer to form agarwood. To solve this problem, we developed a procedure named secondary agarwood-inducing, as showed in Figure 3.

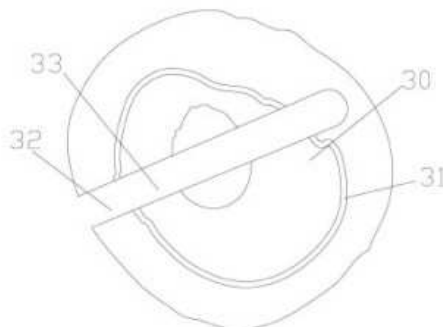


Fig. 3 The procedure for secondary agarwood-inducing

The primary agarwood-inducing area is divided into the peripheral agarwood-inducing layer (31) and the inner agarwood-inducing area (30). After the peripheral agarwood-inducing layer but before the protective layer was formed, a blind hole (32) was drilled into or through the primary agarwood-inducing area at the level of 1.5 m above and 0.5 m under the infusion hole, respectively, thus forming the secondary agarwood-inducing area (33).

#### Qualitative identification of agarwood

According to *Chinese Pharmacopoeia* 2010 Edition (1) [12], Thin Layer Chromatography (TLC) was employed for qualitative identification for obtained resinous wood treated by Modified Kit, with the standard medicinal agarwood as the positive control (offered by National Institute for the Control of Pharmaceutical and Biological Products).

#### Quantitative identification of agarwood

As the Alcohol Soluble Extractive Content is the sole indicator for quantitative identification of medicinal agarwood, according to the appendix XA of *Chinese Pharmacopoeia* 2010 Edition (1) [12], and a core indicator for its quality grading [13], it was employed for quantitative identification for the resinous wood harvested three-, six-, 12-, 16-, and 18-month after treatment by Modified Kit, respectively ( each sample was detected for three times).

## RESULTS AND DISCUSSION

Agarwood that meets Chinese Pharmacopoeia standard was formed in *A. sinensis* six month after the employment of Modified Kit, as shown in Figure 4.



Fig. 4 Trunk cross sections six month after treatment with Modified Kit

**Qualitative identification of agarwood**

The TLC fingerprint profiles of the resinous wood treated by Modified Kit and the standard medicinal agarwood are shown in Figure 5. The fluorescent speckles of the resinous wood took an identical color at the corresponding positions of standard medicinal agarwood under UV<sub>254nm</sub> and UV<sub>365nm</sub>. These results demonstrate that the resinous wood obtained by Modified Kit was agarwood.

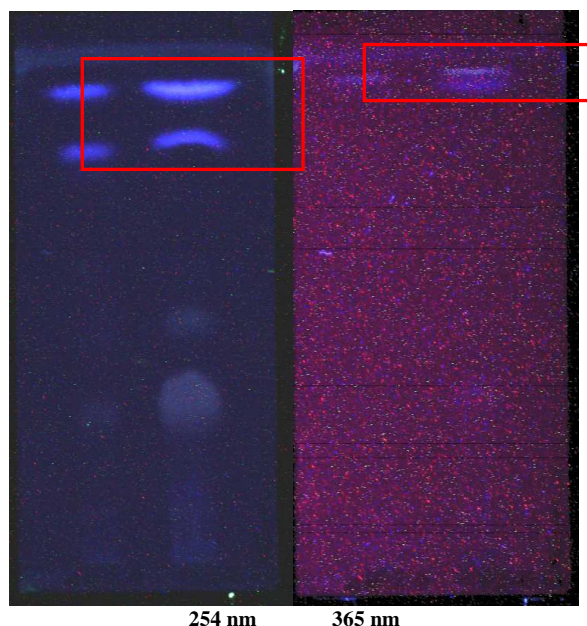


Fig. 5 TLC chromatogram of ethyl ether extracts. Kit

The left column in each picture represents the standard medicinal agarwood, and the right column represents the resinous wood treated by Modified.

**Quantitative identification of agarwood**

According to *Chinese Pharmacopoeia* 2010 Edition (1) [12], using ethanol as the solvent, the Alcohol Soluble Extractive Content (ASEC) of medicinal agarwood is required to be no less than 10%, by Hot-dip Method under the Determination of the Alcohol Soluble Extractive Content (appendix XA).

The agarwood was formed in the whole *aquilaria* trunks three-month after the treatment by Modified Kit, and the average ASEC in all agarwood obtained at the six-month met *Chinese Pharmacopoeia* standard, and reached the peak value of 43.9% at the 16-month, then showed a downward trend. These results indicate that 16-month after treatment by Modified Kit is appropriate for harvest. Table 3 shows the average ASEC of agarwood obtained three-, six-, 12-, 16-, and 18-month after treatment, respectively.

Table 3 The average ASEC of agarwood obtained three-, six-, 12-, 16-, and 18-month after treatment by Modified Kit, respectively

Sample code	Treatment time (month)	The average ASEC (%)
1	3	3.27
2	6	23.09
3	12	35.74
4	16	43.90
5	18	33.08

**DISCUSSION**

Agarwood formation in natural environment usually takes more than 10 years; Artificial Agarwood-Inducing Techniques, such as axe chopping method, can only be applied until the *Aquilaria* trees grow mature, which generally takes a few years, and then it has to take another few years for the agarwood to meet the *Pharmacopoeia* standard [2]; Nevertheless, by adopting Modified Kit, including the transfusion needle and vessel, inducer and procedure (for primary and secondary whole tree agarwood-inducing process), agarwood that meets *Pharmacopoeia* standard could be obtained only six months after treatment, thus significantly shortening the Production Period.

Moreover, comparing to about 15% of the average ASEC obtained in agarwood treated by traditional "Infusion Technique" [3], the Modified Kit showed a relative improvement.

### CONCLUSION

The Modified Kit, including the transfusion needle and vessel, inducer and procedure (for primary and secondary whole tree agarwood-inducing process), is an efficient method for agarwood -inducing. The plant transfusion needle can prevent the fluid outlets from being blocked, and the fluid from oozing out of the trunks, thus improve the infusion efficiency. The design of the infusion vessel is helpful to make the clogging in transfusion needles or tubes easily detected, then quickly handled. Adopting the procedure for primary and secondary whole tree agarwood-inducing, combined with the inducer, the quality of obtained agarwood could be significantly improved and the Production Period shortened. Moreover, as the inducer is mainly composed of *Schizophyllum commune*, an edible mushroom parasitized in *A. Sinensis*, and other food materials, the obtained agarwood is free of chemical residues and the extensive damage leading to decay or death to the barks and trunks usually brought by phytohormones and chemical agents would not happen.

The Modified Kit, if being widely popularized and applied, will produce billions of dollars of economic benefits, create enormous employment and income-generating opportunities, and promote environment protection and forestation.

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