2015 Wood Chemistry – 9th Week Antifungal Effects of Essential Oil (Ⅰ)

TA: Seong-Min Cho

Thin-Layer-Chromatography (TLC) Bioassay

Essential oil, also called as phytoncide, is volatile substances released from trees and plants as protective mechanisms against harmful insects, animals and microorganisms. It is synthesized as secondary metabolites of photosynthesis and well known for antibacterial and antifungal agent. Moreover, it has been reported that essential oil has various effects, such as reducing stress response, an anti-oxidative effect and the improvement of various disorders including accelerated aging, allergies, multiple sclerosis, and Parkinson disease. The major components of essential oil are terpenoids, known as volatile flavors of trees and plants.

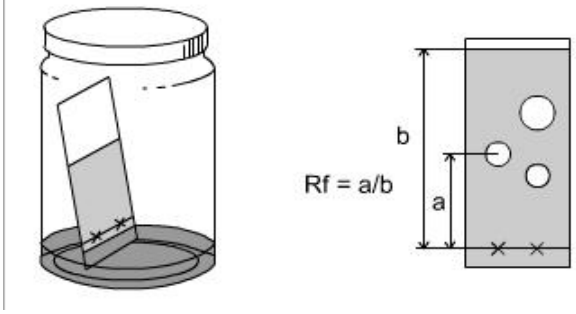
In our experiments today, we assess several tree essential oils by thin-layer-chromatography (TLC) bioassay and estimate which components have the potency of antifungal activity.

1. Materials

① Tree Essential Oil
  Group 1: *Cryptomeria japonica*
  Group 2: *Chamaecyparis obtusa*
  Group 3: *Abies holophylla*
② Fungi (Dermatophytes): *Epidermophyton floccosum*, *Trichophyton rubrum*
③ Culture Media: SDA (sabouraud Dextrose Agar) media
④ Equipment: Autoclave, Stationary-incubator, Clean bench, GC/MS

2. Methods

① Prepare sterilized 200 mL of SDA media (Auto-clave at 121℃, 15 min).
② Fractionate the crude essential oil by TLC.
- Dissolve 100 mg of essential oil in 10 mL of ethyl acetate.
- Load the sufficient quantity of dissolved sample on TLC plate.
- Develop with hexane and ethyl acetate solution (8:1, v/v).
- After vaporize the mobile phase, cut off the ends of plate.
- Soak the strip of TLC plate in aqueous phosphomolybdic acid solution to observe separated sample.
- Group several fractions together by color development.



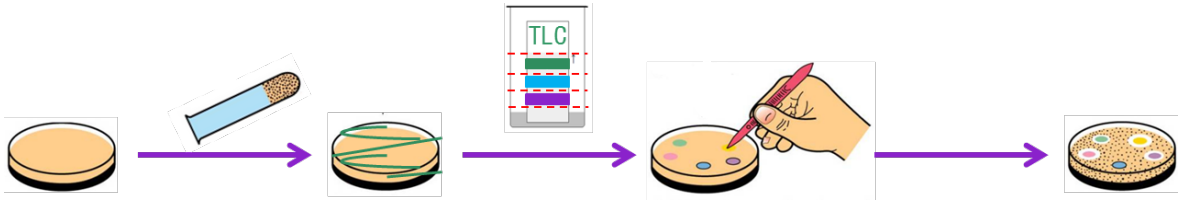
③ Cut the grouped TLC plate (contains fraction of essential oil) into a proper size for bioassay.

④ Pour the hot liquid SDA media into Petri Dish and cool down to solidify it at clean bench.

⑤ Prepare the spore suspension of pre-cultured strain by doing dilution with sterile water. (McFarland Turbidity Standard No. 0.5, OD_{600}=0.132, 1.5×10^8 CFU/mL)

⑥ Inoculate and smear the prepared spore suspension on the solidified SDA media.

⑦ Put and fix the grouped TLC plate piece on the smeared SDA media.



⑧ Cultivate at 28℃ for 1 days and check the formation of clear zone.

⑨ Scrape the silica off the remaining TLC plate piece.

⑩ Extract the fractionated essential oil from silica by dissolving and vortexing in 5 mL of ethyl acetate.

⑪ Sample the ethyl acetate solution by hydrophobic filters and GC/MS analyze it.

§ Report

※ Describe data of 9th week and 10th week together, and should include results of other groups.

※ Report should be written by MS words (10 points, line spacing 1) or Hancom office (10 points, line spacing 120)

※ Report must be taken in the following order (in Korean or English): 1. Introduction, 2. Materials and Methods, 3. Results and Discussion, 4. Conclusions, 5. References

※ Please define terpenoid and show its biosynthesis pathway. And briefly assort various type of terpenoid compounds in terms of mono-, di-, sesqui- and so on.

※ Please describe the principle of GC/MS.

※ Assignment should be appended to report. (If you copy and paste, you can not get a grade)

※ Inquires: ① Wood Chemistry Lab (6203) Seong-Min Cho, ② csmin93@snu.ac.kr ③ 010-6623-5449