Investigation on the Some Active Compounds of the Agarwood Oil

(Aquilaria agallocha Roxb)

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သစ်မှာပါဝင်သောဓါတုပါပါပေးအချိန်နှင့် သုံးဓါတုပါပါများ၏ ဂုဏ်သတ္တာများနှင့်အစွမ်းထက်ပုံတို့ကိုစူးစမ်းလာခင် စုမင်သန်း၊ သုံးသန်းလက်ထောက် ၂ခါလွင်၊ သုံးသန်အရာရှိသီတာချိန်၊ သုံးသန်လက်ထောက် ၂စာတမ်းအကျဉ်းကချင်ပည်နယ်၊ မွစ်ကီးနားမိုနှင့် တနသာရီတိုင်းဒသာမိတ်မိတ့်နယ်မှ ရရှိသော သစ်မှာ (၂)မျိုးတို့တွင်ပါဝင်သော ဓါတုပါပါများ (၃)မျိုးနှင့်အတူ အေချအမည်မသိဓါတုပါပါများ (၂)မျိုးပါဝင်ကာင်း တွေ့ရှိရပါသည်။ ကချင်ပည်နယ်မှ ရရှိသော သစ်မှာ နပူဖသလင်း၊ ဆိုင်ကလိုပန်တနာနှင့် အက်ဂျင်ဖူရမ်ဟူသော ဓါတုပါပါများ (၃)မျိုးနှင့်အတူ အေချအမည်မသိ ဓါတုပါပါများ (၂)မျိုးပါဝင်ကာင်း တွေ့ရှိရပါသည်။ ထိုအေပ် သုံးဓါတုပါပါများ (၃)မျိုး၏ ပါဝင်မှပိုက်မှုကို "စ" စံဖွေထွက်ချိန်နှင့် နှင့်ယှဉ်ထားပါသည်။
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Abstract

The study was carried out to find the three active compounds of essential oils of agarwood (*Aquilaria agallocha* Roxb.) collected from Myeik Township and Myitkyinar Township by using High Performance Liquid Chromatograph analysis. The *A.agallocha* oil from Myeik contained Naphthalene, other (2) unknown compound, Cyclopentanone and agarofuran. Also the *A.agallocha* oil from Myitkyinar included Cyclopentanone, Naphthalene and Agarofuran compound. The characteristic of compound constituents are generally measured by comparing the chromatograms’ retention time with that of reference standard. So it was observed that these compounds (cyclopentanone, Naphthalene and agarofuran) of agarwood oil were found to be lower in Myeik Township than in Myitkyina Township. Moreover there are revealed that three compounds of Agarwood oil’s properties and activities. On the basic of above fact it may be concluded that *A.agallocha*, may be utilized as a source of naphthalene, cyclopentene and agarofuran respectively. This may indicate that the micro flora is of great importance in production of specialized type of agarwood for best quality agar oil. However, they may exist variants or eco-types within the agarwood plant species. It natural variant or eco-type exist with the plant species, the fungal pathogens might be host type specific or variant specific. Therefore, identification of natural variant or eco-type and the specific host-pathogen relationship under different ecological conditions are expected to give due for unraveling the secret of agar formation.
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Investigation on the Some Active Compounds of the Agarwood Oil (Aquilaria agallocha Roxb)

1. Introduction

Agar is a valuable aromatic oleoresinous deposit found in the stems of Aquilaria agallocha Roxb (large evergreens native to southeast Asia). Belonging to the Aquilaria genus, Thymelaeaceae family. It is sometimes referred to as “liquid god” or “wood of the gods”. Aquilaria grow very fast, and start producing flowers and seeds as four years old. Experiment is conducted on five years old. Among the fifteen species of Aquilaria trees, only Aquilaria agallocha and Aquilaria malaccensis are planted in Myanmar, especially in Myitkyina Township and Myeik Township.

As the international demand for agar increases, agarwood is becoming rare and difficult to find. The resinous agarwood product is only found naturally in less of tree, with a still imprecisely understood combination of wounding, vectors of infection (bacterial infection, fungus) and resinous response (to external and internal causal factors) producing the formation of agar wood. When they become infected with a type of mold. Prior to infection, the heartwood is relatively light and pale colored, however as the infection progresses, the tree produces a dark aromatic resin in response to the attack, which results in a very dense, dark, resin embedded heartwood.

In Myanmar, regional names were Thitmwetha (Khanti and Mawlike), Lawpan (Rangon and lower Myanmar), Akyaw (Taninthayi Division), TheinNaing (Teatain region). Agar oil is used primarily for medicine, perfume and incense. Agar woodchips are meant to be used as incense. A strong connection exists between use, religion and curative properties, and elaborate traditional and religious ceremonies are known from around the world.

The chemical compositions of the agar oil depend not only on Aquilaria species, but also on production methods are being considered.

Therefore the objecting of the study is to investigate on some chemical properties and active properties of the different agar oil in the market should be initiated in order to understand.

2. Objectives

1. To investigate the main compounds of the agarwood oil and their activities
2. To refer the illegal oil of the agarwood oil
3. To utilize these compound upon their activities

3. Literature Review

Artificial induction of agar in live tree is possible. Takes 8-20 years for agar development.

The chemical constituents of agar wood oil (*Aquilaria crassna*) were analyzed by gas chromatography coupled to mass spectrometry (GC-MS). Extraction was operated at various temperatures (80°C, 100°C and 120°C). The agarwood oil extracted at 80°C and 100°C have 4 major chemical compounds. The principal compounds at extraction temperature of 120°C were found to be *agarospirol*, *junipene*, *palmitic acid*, *myristic acid*, *pentanone* and *benzylactone*.

The agar oils (*Aquilaria agallocha* Roxb.) obtained from healthy, naturally infected and artificially screws wounds. They were to find out the differences in composition by (GC-MS). Natural healthy agar contained *octacosane*, *naphthalene*, *caryophylene oxide*. Natural infected plants agar contained *cycloheptane*, *naphthalene*, *caryophylene oxide*. Artificially screw injected plants agar contained *dioctyl phthalate*, *hexadecanoic acid naphthalene*, *aristolene*.

The chemical constituent of essential oil from Chinese eaglewood (*Aquilaria sinensis* Lour.) was extracted by water-steam distillation and by GC-MS. The major sesquiterpenes were identified *Agarofuran*, *kusunol*, *agarospirol*, *naphthalene* and *baimuxifuranic acid*.

Essential oils are not the same as perfume oils or fragrance oils because artificially created fragrances contain artificial substances or are diluted with carrier oils and do not offer the caliber of therapeutic benefits that essential oil offer.

Resinous wood is used as incense, for medicinal purposes, and pure resin in distilled form is used as perfume and perfume component. Essential oils are generally extracted by distillation. Other processes include expressions, or solvent extraction. They are used in perfumes, cosmetics and bath products, for flavoring food and drink, and or scenting and house hold cleaning products. There are currently three types of plants which are derived from resin; myrrh, agar and frankincense. International trade names include agarwood, aloewood and eaglewood and oudh (Arabic), jin-koh (Japanese), gaharu (Malay), tram huong (Vietnamese), maikhedsana (Lao) and Chen xiang (Chinese) (Barden et al. 200). There are many names for this resinous wood, including agar, agarwood (India), aloewood (Thailand), kalamabak (Cambodia) and eaglewood.

Traders around the world have quote prices for pure agar oil as high as USD 30000/kg and grade two oil costs approximately USD 15000/mg. In February 2001, the PNGFA introduced pricing guideline (Table 2). Demand for agarwood and agarwood oil in Myanmar, a raw agarwood found prices ranging from US$ 1000 to 2000 per kilogram and agarwood oil varied between US$ 2000 to 4000 per kilogram with the grades. Grading is a agar subjective and complicated process based on size, color, fragrance strength, longevity, shape, weight, flammability and resin content. The allocation of grades varies from country to country and from buyer to buyer. Currently there are six grades of wood, super A, A, B, C, D and Reject as presented in Table 1. The agarwood oil of *Aquilaria agallocha*has been one’s liking in Europe. The consumer market for agarwood is well developed in the UAE as a “Brand Name”.
Table 1. Guidelines for grading agarwood based on size, shape and weight of wood. (Source: Eaglewood in Papua New Guinea)

<table>
<thead>
<tr>
<th>Grading on colour</th>
<th>Heavy irregular shape</th>
<th>Heavy regular shape</th>
<th>Light large pieces</th>
<th>Heavy thick chips</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black shiny</td>
<td>Super A</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>Mixture of dark black &amp; chocolate brown</td>
<td>B</td>
<td>B</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>Mixed colour (pale lack or chocolate brown)</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>Brown</td>
<td>D</td>
<td>D</td>
<td>D</td>
<td>D</td>
</tr>
<tr>
<td>Pale yellow or tan brown</td>
<td>D mostly rejected</td>
<td>D mostly rejected</td>
<td>D mostly rejected</td>
<td>D mostly rejected</td>
</tr>
<tr>
<td>White</td>
<td>Rejected</td>
<td>Rejected</td>
<td>Rejected</td>
<td>rejected</td>
</tr>
</tbody>
</table>

Table 2. PNGFA guideline on the minimum prices (Source: RMAP Working Papers)

<table>
<thead>
<tr>
<th>Grade of agarwood</th>
<th>Value USD/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Super A</td>
<td>560</td>
</tr>
<tr>
<td>A</td>
<td>420</td>
</tr>
<tr>
<td>B</td>
<td>280</td>
</tr>
<tr>
<td>C</td>
<td>140</td>
</tr>
<tr>
<td>D</td>
<td>14</td>
</tr>
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</table>

4. Materials and Methods

4.1. Materials

*Aquilaria agallocha* resinous woods were collected from Myeik Township of during November, 2010 and Myitkyinar Township of during March, 2011.
4.2 Methods

4.2.1 Extraction of essential oil

The resinous wood was dried until moisture content was 3% dry basis before ground. The grinded materials were soaked in 5L distilled water up to 14 days and filtered them separately. The filtrate water mixture was placed with Clevenger-type apparatus individually for isolation of oils by hydrodistillation. After 72 hours essential oils were collected separately and dried over anhydrous sodium sulfate. The oils were then stored in sealed container under refrigeration prior to analysis.

4.2.2 Preparation of standards (OR) Standard solution

Each of the stock solutions Naphthalene, Cyclopentanone and Agarofuran were dissolved in mobile phase as 0.5 mg/ml concentrations. The solutions were filtered through a 0.45µm membrane filter. The three standards were freshly and immediately injected to HPLC column.

4.2.3 Sample preparation

5mg of the essential oil samples were weighed and transferred into 10ml volumetric flask and dissolved in 10ml mobile phase, to prepare 0.5mg/ml sample solution. Then, all samples were filtered with 0.45µm membrane filter.

4.2.4 HPLC analysis

The two types of *A.agallocha* essential oil from different locations were analyzed by HPLC by using UV detector.

For the HPLC analysis, oils were mixed with AcoEt(4ml) overnight at room temperature. The extract was concentrated to dryness. The residues was dissolved in MeOH at 1mg/ml and analyzed by HPLC by using a LC-20AT solved delivery module, DGU-20A online Degasser and Shimadza column oven CTO-20A using LC system column. The HPLC column was eluted with water-methanol at a flow rate of 0.8ml/min. Ultraviolet detection was performed at 254nm with a Shimadza multi-function ultra-violet visible spectrophotometric detector SPD-20A.

Peak were identified by comparing retention times with those of standard samples which were isolated from the extract of withered or artificially wounded wood of *Aquilaria* and identified by 'H-NMR and C-NMR, and were quantified by calculating according to the calibration curve of the sample.

4.2.5 Identification of the compound

Compound identification was done by comparing the standard curve. Percentage composition was computed from HPLC column peak area on Chromatogram.
5. The activity of three components

5.1 Naphthalene (tar camphor) C_{10}H_{8}

![Image of naphthalene molecule]

5.1.1 Properties:

Molecular mass 128.17 g mol^{-1}; White crystalline, volatile flakes; strong coal-tar odor. Soluble in benzene, absolute alcohol and ether; insoluble in water. Sp.gr. 1.145 (20/4C); m.p 80.26 °C; b.p. 217.96 °C; flash point 176 F (79-87 °C). Sublimes at room temperature. Autoignition Tem. 979 °F (525 °C). Combustible.

5.1.2 Hazard:

Flammable, sensitizer, possible carcinogen. Dust can form explosive mixtures with air. Toxic by inhalation. Tolerance, 10 ppm in air.

5.1.3 Uses:

Intermediate (phthalic anhydride, naphthol, “Tertralin”, “Decalin”, chlorinated naphthalenes, naphthyl and naphthol derivatives, dyes); moth repellent; fungicide; smokeless powder; cutting lubricant; synthetic tanning; preservative; textile chemicals; emulsion breakers; scintillation counters; antiseptic.

Naphthalene is used mainly as a precursor to other chemicals. The single largest use of naphthalene is the industrial production of phthalic anhydride, although more phthalic anhydride is made from o-xylene. Other naphthalene-derived chemicals include alkyl naphthalene sulfonate surfactants, and the insecticide 1-naphthyl-N-methylcarbamate (carbaryl). Naphthalenes substituted with combinations of strongly electron-donating functional groups, such as alcohols and amines, and strongly electron-withdrawing groups, especially sulfonic acids, are intermediates in the preparation of many synthetic dyes. The hydrogenated naphthalene estetrahydronaphthalene (tetralin) and decahydronaphthalene (decalin) are used as low-volatility solvents. Naphthalene is also used in the synthesis of 2-naphthol, a precursor for various dyestuffs, pigments, rubber processing chemicals and other miscellaneous chemicals and pharmaceuticals.
Naphthalene sulfonic acids are used in the manufacture of naphthalene sulfonate polymer plasticizers (dispersants), which are used to produce concrete and plasterboard (wallboard or drywall). They are also used as dispersants in synthetic and natural rubbers, and as tanning agents (suntans) in leather industries, agricultural formulations (dispersants for pesticides), dyes and as a dispersant in lead–acid battery plates.

Naphthalene sulfonate polymers are produced by reacting naphthalene with sulfuric acid and then polymerizing with formaldehyde, followed by neutralization with sodium hydroxide or calcium hydroxide. These products are commercially sold in solution (water) or dry powder form.

5.2 Cyclopentanone (hepta methylene; suberane) C₅H₈O

5.2.1 Properties

Clear, Colorless liquid soluble in alcohol; slightly soluble in water. Sp.gr.0.809; b.p130.6 C; m.p -58.2 C; aniline equivalent -6; flash point below 26 C)

5.2.2 Hazard:

Moderately toxic; narcotic action by inhabitation flammable, dangerous fire risk.

5.2.3 Uses:

As a fragrance. It is a versatile synthetic intermediate, being a precursor to cyclopentobarbital.

5.2.4 Related compounds:

Cyclopropane.
5.3  Agarofuran (Furane, Divinylene oxide) C₄H₄O

![Diagram of Furan]

5.3.1  Properties:

Molecular mass 68.08g mol⁻¹; Clear, mobile liquid, ether like odor; soluble in common organic solvents, including alcohol, ether and acetone, but insoluble in water. m.p 85.6 °C; b.p. 31.3 °C; flash point -69 °C. Explosive limits: lower 2.3%, upper 14.3% @ 20 °C. Autoignition Tem. 390 °C.

5.3.2  Hazard:

Toxic and may be carcinogenic.

5.3.3  Use:

It is used as a solvent as well as in the synthesis of furfural and other organic compounds. It is converted to more important solvent, tetrahydrofuran by hydrogenation. Nitro-substituted furan derivatives are used as biocides or fungicides to inhibit bacterial growth. Sulfur-substituted furan derivatives are used as flavouring agents. Furfural (Furfuraldehyde), a derivative of furan, is a viscous, colorless liquid that has a pleasant aromatic odor; upon exposure to air it turns dark brown or black; boils at about 160 °C; soluble in ethanol, ether and somewhat in water. It is commonly used as a solvent. Furfural is the aldehyde of pyromucic acid; it has properties similar to those of benzaldehyde. It is prepared commercially by dehydration of pentose sugars obtained from cornstalks and corncobs, husks of oat and peanut, and other waste products. The major application of fufural is being used as a feedstock for furfuryl alcohol. The most commercial quantity of furfuryl alcohol is used in the production of thermosetting furan resin and furan cement, strong adhesive, in which the furan ring is an integral part of the polymer chain providing highly resistance to chemicals. Furfural is used as a solvent for refining lubricating oils and butadiene extraction. It is used as a fungicide and weed killer. It is used in the production of tetrahydrofuran (THF), saturated form of furan. THF is one of the most polar ethers. It is used as an important industrial solvent recognized for its unique combination of useful properties. It is a colorless, volatile cycloaliphatic (5-membered) ether with a characteristic odor; boiling point at 66 °C; soluble in water and organic solvents. THF is unstable...
at room temperature due to possibility of peroxide formation; stabilized sometimes with BHT. Its unhindered oxygen atom carries two unshared pairs of electrons - a structure which favors the formation of coordination complexes and the solvation of cations. THF is made also by eliminating water from 1,4-butanediol. THF is used as an useful chemical intermediate especially as a starting materials for the preparation of nylon.

5.4 Safety

Furan is found in heat-treated commercial foods and it is produced through thermal degradation of natural food constituents. Notably, it can be found in roasted coffee, instant coffee, and processed baby foods. Exposure to furan at doses about 2000 times the projected level of human exposure from foods increases the risk of hepatocellular tumors in rats and mice and bile duct tumors in rats. Furan is therefore listed as a possible human carcinogen.

6. Results and Discussion

The A.agallocha oil from Myeik contained Naphthalene and other (2) unknown compound, Cyclopentanone and agarofuran. Also the A.agallocha oil from Myitkyinar contained Cyclopentanone, Naphthalene and Agarofuran group.

The characteristic of compound constituents are generally measured by comparing the chromatograms’ retention time with that of reference standard. So it was observed that these compounds (cyclopentanone, Naphthalene and agarofuran of agar-wood oil were found to be lower in Myeik Township than Myitkyina Township. On the basic of above fact it may be concluded that A.agallocha may be utilized as a source of naphthalene, cyclopentene and agarofuran respectively.

This may indicate that the micro flora is of great importance in production of specialized type of agarwood for best quality agar oil. However, they may exist variants or eco-types within the agarwood plant species. It natural variant or eco-type exist with the plant species, the fungal pathogens might be host type specific or variant specific. Therefore, identification of natural variant or eco-type and the specific host-pathogen relationship under different ecological conditions are expected to give due for unraveling the secret of agar formation.

7. Conclusion and Recommendations

We analyzed some chemical properties and active properties of the different agar oil (Grade.2 agarwood extractive) from Myitgyina Township and Myeik Township such as the marketing. Therefore the different production methods and the different extraction methods should be continuously tested for modified essential oil.
Over harvesting and habitat-loss threatens some populations of agarwood-producing species. Concern over the impact of the global demand for agarwood has thus led to the inclusion of the main taxa on CITES Appendix II, which requires that international trade in agarwood is subject to controls designed to ensure that harvest and exports are not to the detriment of the survival of the species in the wild.

In addition, agarwood plantations have been established in a number of countries. Numerous inoculation techniques have been developed, with varying degrees of success. The newly developed methods of agarwood are providing a new economy. This new economy in rural areas will help many of the poorest people. The sustainable production of agarwood in plantation grown trees eliminates the need to cut old growth forest trees for the resin and will help save this endangered tree from possible extinction. This work is also provides a source of Analysis of agarwood so this magnificent aromatic resin can enjoyed by people throughout the country.

![Graph showing UV and time analysis of agarwood compounds.](image)

**Fig. 1** There are three compounds of agarofuran, naphthalene and cycloantanone are measured by HPLC from Myeik Township.
Fig.2 There are three compounds of agarofuran, naphthalene and cyclopantene are measured by HPLC from Myitkyina Township.

Fig.3 Sample collection from Myeik
Fig. 4  Sample collection from Myintkyina

Fig. 5 Sample
Fig.6. Extraction of essential oil

Fig.7 Preparation of Standard

Fig.8 Sample Preparation for HPLC
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