

ASSESSMENT OF AGARWOOD HYDRODISTILLATION BYPRODUCT FOR
CONSUMABLE POTENTIAL

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Specially made for the love of my life; my parents and family,
To dear self who is always eager to achieve anything in her life even though she
always feels she cannot do it,
You have made it.



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ABSTRACT

Agarwood hydrosol is a by – product of the hydrodistillation process in the extraction of essential oil usually being underutilized although some consumers believe that all components of agarwood have health benefits. To enhance the value of hydrosol in the market, three (3) formulations were designed based on different type of sugar used; F1 (white sugar), F2 (brown sugar) and F3 (white sugar + brown sugar) which hydrosol was used as main ingredient. To determine the sweetened agarwood drink (SAD) characteristics and preferences, this project undergone analyses; sensory, physicochemical, antioxidant, cytotoxicity and enumeration of yeast and mold for shelf – life analysis according to AOAC method and Malaysian Food Regulation (1985). The data summarized that drink obtained dislike perception. The characteristics of SAD was acidic (pH 4.42), viscosity (2.14 m.Pa.s), ash (0.31 g), protein (1.23g), carbohydrate (9.26 %) and sugars (12.28 %), no fat, contain sodium, potassium, zinc, magnesium and calcium at the same time no heavy metals were detected. However, low antioxidant properties exerted on drinks; DPPH activity (32.54%), FRAP (0.73 mmol Fe²⁺ E/L), TPC (0.2 mg GAE/ml) while TFC (2.28mg RE/ml). Weak correlation between DPPH, TPC and TFC which R² = 0.49 and 0.56 respectively and no correlation between FRAP to TPC and TFC. Cytotoxicity analysis on brine shrimp was 100% mortality. Shelf–life analysis showed no yeast or mold growth in drinks. Overall, formulations made to SAD did not obtained panellist acceptability but increase the product physicochemical properties. This drink lack of antioxidant value, and brine shrimp assay did not suitable for drink toxicity analysis because control drink also resulted 100% mortality. Addition of preservatives in drink formulation prolong the shelf life of drink to more than 1 ½ years storage.

ABSTRAK

Hidrosol gaharu adalah produk – dari proses hidrodistilasi dalam pengekstrakan minyak pati yang biasanya kurang digunakan walaupun sebilangan pengguna percaya bahawa semua komponen kayu gaharu mempunyai manfaat kesihatan. Untuk meningkatkan nilai hidrosol di pasaran, tiga formulasi (3) direka berdasarkan jenis gula yang digunakan; F1 (gula putih), F2 (gula perang) dan F3 (gula putih + gula perang) manakala hidrosol digunakan sebagai bahan utama. Untuk mengenalpasti ciri dan pilihan kepenggunaan minuman gaharu berperisa manis, projek ini menjalani analisis; deria, fizikokimia, antioksidan, kesitotoksikan dan analisis jangka hayat mengikut kaedah AOAC dan Peraturan Makanan Malaysia (1985). Ringkasan data menunjukkan bahawa minuman tidak digemari oleh ahli panel. Ciri-ciri minuman gaharu berperisa adalah berasid (pH 4.42), kelikatan (2.14 m.Pa.s), abu (0.31 g), protein (1.23g), karbohidrat (9.26%), tidak ada lemak, mengandungi natrium, kalium, zink, magnesium dan kalsium serta tiada logam berat. Walau bagaimanapun, sifat antioksidan dalam minuman adalah rendah; Aktiviti DPPH (32.54%), FRAP (0.73 mmol Fe²⁺E/L), TPC (0.2 mg GAE/ml) manakala TFC (2.28mg RE/ml). Terdapat korelasi yang lemah antara DPPH, TPC dan TFC yang masing-masing bernilai R² = 0.49 dan 0.56 dan tidak ada hubungan korelasi antara FRAP dengan TPC dan TFC. Analisis kesitotoksikan terhadap udang air garam adalah 100% kematian. Analisis jangka hayat tidak menunjukkan pertumbuhan yis dan kulat dalam minuman. Secara keseluruhan, formulasi yang dibuat bagi minuman gaharu berperisa manis tidak diterima oleh ahli panel tetapi formulasi telah meningkatkan sifat fizikokimia minuman. Minuman ini kekurangan nilai antioksidan, dan ujian udang air garam tidak sesuai untuk analisis ketoksikan minuman kerana sampel minuman kawalan juga mengakibatkan 100% kematian. Penambahan bahan pengawet dalam formulasi minuman memanjangkan jangka hayat minuman hingga lebih dari 1 ½ tahun penyimpanan.

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LIST OF SYMBOLS AND ABBREVIATIONS

<i>g</i>	-	gram
<i>mg</i>	-	milligram
<i>ml</i>	-	mililiter
mEQ	-	milliequivalent
<i>l</i>	-	liter
AUH	-	autoclave hydrosol
ANOVA	-	Analysis of Variance
DPPH	-	2, 2 – diphenyl – 1 – picrylhydrazyl
FRAP	-	Ferric reducing antioxidant power
PET	-	polyethylene terephthalate
PP	-	polypropylene
RH	-	raw hydrosol
RTD	-	Ready – to – drink
WOH	-	without hydrosol



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PERPUSTAKAAN TUNKU TUN AMINAH

CHAPTER 1

INTRODUCTION

1.1 Background of study

Agarwood is also known as aloeswood, eaglewood, oud, chenxiang and jinkoh according to different country. In Malaysia, agarwood is called as gaharu. Agarwood is highly valuable resin heartwood that contain fragrant which can be found in Aquilaria species of *Thymelaeacea* family (Ismail *et al.*, 2014). There are variety types of agarwood species includes *A. crassna*, and *A. sinensis* while agarwood species that mostly found in Malaysia is *A. malaccensis*. Agarwood is developed in resulting of plant defense mechanism from infection and injury. The mechanisms might due to wounding, insertion of microbial agarwood plant (from inoculation process) or response of the tree towards chemical stress (Hashim *et al.*, 2016).

There are many techniques have been used to extract agarwood essential oil such as distillation; hydrodistillation or steam distillation, solvent extraction, and supercritical fluid extraction (Naef, 2011). The most frequently technique that being used in extraction of agarwood essential oil is hydrodistillation because it is safe to conduct by human and environmentally friendly as the plant material is completely immersed in boiling water at the same time the surrounding water acts as a barrier to protect the oil from overheating (Sovova & Aleksovski, 2006). The agarwood essential oil is highly demanded because it is widely been used as an incense, perfumeries, and cosmetics.

Other by – products from distillation process that are also utilized is hydrosol. Hydrosol or hydrolates or aromatic waters contains small fraction of essential oils aromatic compounds and several water soluble components gained from plant that undergoes the distillation process (D'Amato *et al.*, 2018). Essentially, hydrosols

contain oxygenated compounds that notable as antimicrobial and antioxidant properties (Shafie *et al.*, 2022) depending on plant species, its origin and the section of the plant extracted (Jakubczyk, Tuchowska, & Janda-Milczarek, 2021). Recent study has found that agarwood have healing properties in aromatherapy and acts as traditional medicine for tropical and oral consumption (Hussein *et al.*, 2019). Generally, hydrosols are used in cosmetics and beauty products such as toners, shampoo, conditioner, perfumeries; body mist, aromatherapy soap or steam baths (Shafie *et al.*, 2022) and currently, several research study has been made on functional of plant hydrosol from herbs or spices hydrosol as food sanitiser (Törnük & Dertli, 2015; Ozturk *et al.*, 2016). Hydrosols are also have been consumed as beverage in Turkey region many years ago (Sağdıç & Özcan, 2002) as it is said that hydrosols are cheap to produce and less toxic to human health, compared to essential oils. Persian culture commonly used hydrosol as functional beverages for mental and neurological disorders such as antifatigue, antianxiety, anticonvulsant and analgesics for headache (Hamed *et al.*, 2017b).

Nevertheless, limited study on consumption of agarwood hydrosol as drink products has been reported until now. Therefore, the purpose of this study is to produce new edible ready – to – drink products made from hydrosol of agarwood with certain heat treatment that safe for human consumption and beneficial for health. Enhancement of hydrosol drink can be made by addition of permissible ingredients according to Malaysian Food Law and Regulation (1985); which the regulation focus on general standard of soft drinks and flavoured drink. Ingredients that commonly used are sugar, additives, preservatives, colorant, vitamins and phytochemicals derived from other sources. White sugar and brown sugar were added sugar that made up from natural sources such as sugarcane or sugar beet. White sugar and brown sugar can provide adequate sweet taste to drinks despite using in small amount compared to synthetic sweetener and sugar alcohols which have 70 % to 100 % sweeter than white sugar. High quantities usage of these sugar in drinks prone to cause gastrointestinal discomfort and diarrhea to consumer (Mooradian, Smith, & Tokuda, 2017). Thus, agarwood drink that added with white sugar and brown sugar suitable to consumer that emphasize with natural sources products usage rather than synthetic sweetener. Heat processing such as hot filling, high temperature shorter time (HTST) pasteurisation or sterilisation primarily being used in drink production to ensure that the drink is safe from microbial contamination and prolong the shelf – life of finished products. Basic

characteristics of packaging materials that suitable for agarwood drink are the materials can withstand to high temperature, as the drink production must be operated in aseptic condition; opaque and low permeability to surrounding; lightweight and economic such as glass and polypropylene (PP).

1.2 Problem statement

Agarwood hydrosol is aromatic water or hydrolates that is produced as by – product from hydrodistillation of agarwood chips to obtain essential oil. According to agarwood industry; Agarwood Technology Plantation (ATP) Sdn. Bhd (unpublished), hydrodistillation of 20 kilograms of agarwood roughly produced ten (10) to twenty (20) litres of hydrosol. Underutilized of hydrosol after hydrodistillation prone to be disposed as effluent by agarwood industry because limited usage of hydrosols in daily lives. Hydrosols commonly being used in cosmetics products and fragrances, but in least amounts. These products are considered as premium, long term applicable and usually being sold in high price. This perception on the ready - made agarwood products may limit the target market for the industry and not economy – friendly. Nevertheless, lack of knowledge on application of hydrosols into beneficial products either consumable or non – consumable tend to make it become wastage and valueless.

Recent study stated that human is tolerable to essential oil, thus hydrosols from plant extract is considered as safe for human consumption and do not carry any health issues (Altinterim, Gulec, & Aksu, 2012). In general, abundance of hydrosols can be developed into other consumable products in terms of food and beverages (F&B) such as sweetened drink. Preliminary study on raw hydrosol stated that hydrosol contain an adequate amount of minerals such as sodium, calcium, magnesium, potassium and zinc but lack of macronutrients such as protein, fat and carbohydrate. Thus, to enhance the physicochemical and antioxidant properties of hydrosol, hydrosol can be formulated with addition of sweetener, preservatives, and fortification with ascorbic acid. This idea may help agarwood industry to vary their market demands ranged from cosmetics, fragrances, and pharmaceutical to F&B at the same time boost up their production profit. Local brands such as Malga have widely produced and marketed the hydrosol – based drink to consumer especially to agarwood enthusiasts which is proved that hydrosol has high demand. In addition, development of new edible products from agarwood hydrosols may attract other consumers from many ranges of economy to

consume the products since the products may give new positive perceptions on agarwood products that will be sold in a cheaper price, contain appropriate nutritional value and can be consume daily.

1.3 Research objective

The objectives for this study were:

- I. To evaluate panellist preferences on sweetened agarwood drink (SAD) using triangle test and hedonic test.
- II. To determine physicochemical properties, antioxidant properties and toxicity of SAD after heat treatment.
- III. To enumerate the presence of yeast and mold in SAD after processing.

1.4 Research scope

There were a few scopes in this study which were:

- I. The hydrosols were supplied by Agarwood Technology Plantation Sdn. Bhd.
- II. The SAD was formulated with addition potassium sorbate, sodium benzoate, colorant and sugar.
- III. Two (2) types of sugar used in drink formulation which were white sugar and brown sugar.
- IV. The formulated SAD was undergone hot filling process.
- V. The packaging used for filling SAD after heat treatment were 150 ml polypropylene (PP) bottle and 150 ml glass bottle.
- VI. Sensory evaluation of the formulated SAD after thermal processing was by using triangle test and hedonic test.
- VII. The physicochemical analysis of SAD was analysed on total sugar content, protein content, carbohydrate content, ash content, minerals content and heavy metals content.
- VIII. The antioxidant analysis of SAD was based on total phenolic content, total flavonoid content, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric reducing antioxidant potential assay (FRAP).
- IX. Toxicity analysis of SAD was based on brine shrimp lethality assay (BSLA).

- X. Enumeration of yeast and mold count was enumerated by Plate Count Agar (PCA) to identify the shelf life of SAD after 1 ½ year of storage.
- XI. The experiment was conducted at Universiti Tun Hussein Onn Malaysia.

1.5 Significance of study

Agarwood hydrosol is one of major by – product from hydrodistillation of agarwood to obtain essential oil. Currently, local farmer only gain profit from essential oil and marketed to be used in perfumeries, incense or folk medicine (Azhar *et al.*, 2021). However, extraction of essential oil needs a long process and time despite the value of essential oil is depends on the grade which is set by agarwood industry. Research study has been stated that highest grade of agarwood can reached up to \$ 13, 000 per pound (Abidin *et al.*, 2015). On the other side, some agarwood trees planted by local farmers does not produce large amount of resin to produce essential oil. This situation creates frustration to local farmer who have spent thousands of moneys to manage the plantation. Other part in agarwood tree such as its leaves can be used to produce new products but further processing is needed and it is costly. Typically, hydrosol usage in non – consumable products are very limited because hydrosol only being used in perfumeries, cosmetics usage and health supplements (Hussein *et al.*, 2019) in small proportions while the remaining will be dumped as waste. This condition imparts the profit of industry as supposedly all by – products especially hydrosol can widely be marketed to obtain maximum profit.

Furthermore, it is believed that hydrosol is edible for human consumption but in necessary manner because it is made up from distillation of plant parts and Ministry of Health also permitted water that undergo distillation process to be consumed such as drinking water. Past study reported that hydrosol may contains all compound in agarwood essential oil but in low concentrations, which it is suitable for developing new products where pure essential oil considered as too strong (Suhaila *et al.*, 2020). In addition, hydrosol can be used as main ingredients in producing new ready – to – drink products at the same time offers variety product options to consumers that seek for nutritious drink with cheaper price but contain appropriate nutritional value for human body. This is because some agarwood enthusiasts claimed that agarwood hydrosol have therapeutic effect to human health especially in cancer treatment but

there are limited literature and findings on chemical component in hydrosol (Azhar *et al.*, 2021). Thus, this project finding provide information on raw hydrosol characteristics, consumer perception on new consumable products from plant hydrosol and validity of the therapeutic value in hydrosol finished products.



CHAPTER 2

LITERATURE REVIEW

2.1 Agarwood formation from *Aquilaria* trees

Agarwood is a resinous hardwood in *Aquilaria* (Thymelaeaceae) trees as a result from defence mechanism during infections or injuries either by chemical or biological methods such as notching, fungal infection, insect or inoculation (Fazila, 2012). Agarwood is also known as gaharu, aloes wood, kritsana and jinkoh according to different regions. Agarwood widely being used in production of incenses, traditional medicines and perfumes. Agarwood – producing species are only can be found around certain areas of India, China and Southeast Asia region while Indonesia and Malaysia are the major countries for agarwood origin (Liu *et al.*, 2013). It is sold in market in the form of chips, blocks and splinters.

When the agarwood is burnt, it will release a pleasant scent of aromatic oils. Agarwood that has high in resin is heavy, hard and brown colour while non – resinous of agarwood has soft and yellow white wood colour (Figure 2.1) (Pojanagaroon & Kaewrak, 2005). Currently, no other substances are similar with agarwood and chemical substitutes that already available cannot imitate the natural aroma of agarwood (Chowdhury *et al.*, 2016).

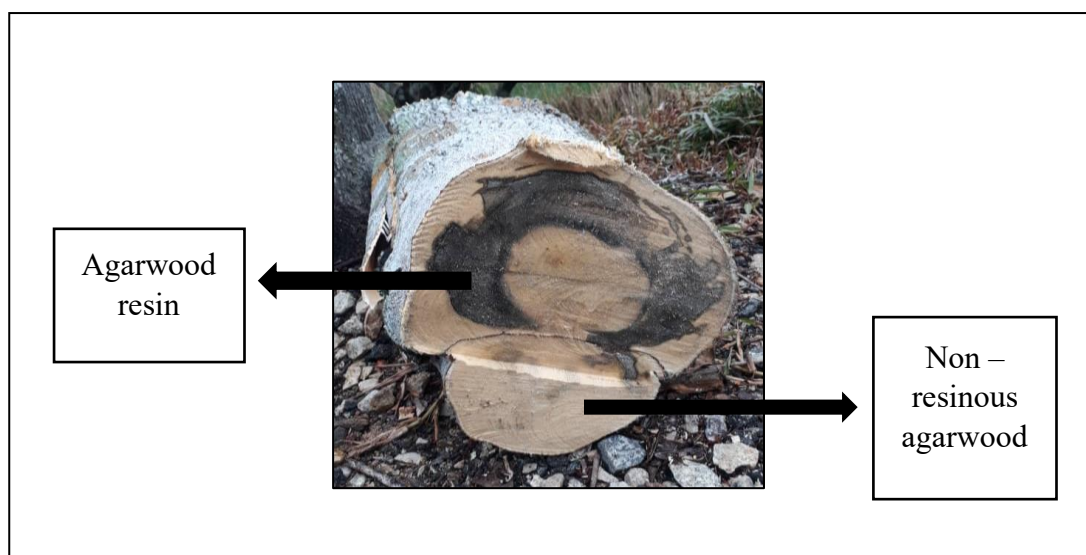


Figure 2.1: Difference between resin and non – resinous of agarwood

According to recent study made by Pojanagaroon & Kaewrak (2005) on *A. crassna* trees for 20 months, agarwood can be formed after mechanical injuries techniques which are gouge with chisels hole with screws, bark with hatchet and puncture with nails. The resin formation changes around the wounding parts varied from pale discoloration after 1 month of wounding, followed by formation of dark yellow – brown discoloration after 3 months, dark – brown formation after 8 to 10 months and black after 20 months.

Besides that, Liu *et al.* (2013) previous study stated that healthy wood of *Aquilaria* trees is white, soft and no scented resins. The agarwood might be formed in natural ways when affected by external factors such as scratching from animals, insect or microbial attacks around wounded or rotting part of *Aquilaria* tree trunk. However, agarwood formation occurred slowly and rarely formed on old *Aquilaria* trees. Table 2.1 below shows different types of method to form resin on agarwood at different species of agarwood. Other agarwood – producing method that already introduced many years ago is inoculation method with foreign media into the wound parts which can form agarwood in a short period of time. Recent study made by Novriyanti *et al.* (2010) reported that chemical media that being used in inoculation was oil, methyl jasmonate and sugar. Liu *et al.* (2013) study on inducing the trees with chemical media named Agar – Wit showed that the resinous wood formed slowly after the inoculation. The brown area that produces as early as 1 week showed that the resin started accumulated inside the *Aquilaria* trunk day by day until it becomes black after 20 months. This efficient method was also being developed in Vietnam but using different

formulation of chemical media mixed with fungi known as agarwood kits (CA – Kits). The main concept of inoculation is by wounding the tree with drilling a hole at certain range of depth at the tree trunk and inoculate the tree with formulated chemical media. Chemical reaction from the chemical media will produce discoloration area which known as agarwood.

Inoculation method by inducing solution that contain fungi into the agarwood tree trunks and branches can also promote agarwood formation. Further research done by Faizal *et al.* (2017) on *Aquilaria malaccensis* tree that inoculated with *Fusarium solani* clarified that fungal inoculation capable to accelerate agarwood production as dark – brown area can be seen around wounding site as early as three (3) months. The formation is due to stress or virulence condition exerted towards the tree when foreign materials is present inside the healthy agarwood tree. This condition activates cell of plants to synthesize secondary bio – chemical substances to defence against the stress (Azren *et al.*, 2019). Previous research by Zhang *et al.* (2014) on *Aquilaria sinensis* that grown in greenhouse which inoculated with *L.theobromae* showed resin formation in stem of trees after six (6) months. The resin was also yield essential oil after hydrodistillation process. This means biological method with aid of fungi capable to enhance production of agarwood other than natural, mechanical and chemical method.

Table 2.1: Method to form resin on agarwood

Method of agarwood formation	Example	References
Natural	lightning strikes, animal gazing, pest, disease infestations	Liu <i>et al.</i> (2013), Azren <i>et al.</i> (2019)
Mechanical	Gouge with chisel hole with screws, Bark with hachet, puncture with nails, wound by axe	Pojanagaroon & Kaewrak (2005), Yan <i>et al.</i> (2019)
Chemical	Inoculation with chemical media, Agar – Wit, CA – kits, oil, sugar, methyl jasmonate	Liu <i>et al.</i> (2013), Blanchette & Heuveling (2009), Novriyanti <i>et al.</i> (2010)
Biological	Inoculation with fungi such as <i>Fusarium</i> sp., <i>Lasiodiplodia</i> sp.	Faizal <i>et al.</i> (2017), Zhang <i>et al.</i> (2014)

2.2 Extraction method to obtain essential oil and hydrosol

The most common extraction method to obtain essential oil and hydrosol of plant is by water distillation (hydrodistillation). Research study made by Tornuk *et al.* (2011) on preparing various plant hydrosols such as thyme, rosemary, black cumin, sage and bay leaf samples are by hydrodistillation method. The plant material was ground and placed into one (1) litre clavenger apparatus set, added with half volume of distilled water and undergo distillation for 1 hour. The essential oil was produced and separated through cooling funnels while hydrosols were collected and covered with sterile bottles until use. This techniques was followed by Ozturk *et al.* (2016) research study with different types of spices. Table 2.2 shows comparison between hydrodistillation method and steam distillation method that can produced essential oil and hydrosol at the same time.

Table 2.2: Comparison between hydrodistillation method and steam distillation method
(Kahar *et al.*, 2021)

Method	Hydrodistillation	Steam distillation
Principle	Plant material immersed completely in water	Plant material not completely immersed in water
Technique	Water surrounds the plant material	Steam surrounds the plant material
Level of pollution	Does not pollute products especially food	Does not pollute products especially food
Condition	No overheating and charring	Possible to overheating and burnt
Economy	Economic	Economic
Usage	Industrial scale	Industrial scale
Products	Produce essential oil and hydrosol	Produce essential oil and hydrosol

Another previous study made by Zheljzakov, Astatkie, & Schlegel (2014) on extraction of coriander fruits to obtain essential oil was also by using hydrodistillation techniques on Florentine apparatus but varied on distillation times which were 1.25 minutes, 2.5 minutes, 5 minutes, 10 minutes, 20 minutes, 40 minutes, 80 minutes, 160 minutes and 240 minutes. The coriander sample was crushed with water by using a blender for hydrodistillation to avoid any essential oil losses (Topalov, 1962). Figure 2.2 displayed hydrodistillation process to extract essential oil and hydrosol. Recently, research made by Samadi *et al.* (2017) used hydrodistillation clavenger method to extract essential oil from *Aquilaria malaccensis* leaves. Dried leaves were grinded and

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