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SYNTHESIS AND CHARACTERIZATION OF ACTIVATED CARBON FROM AGARWOOD (*Aquilaria malaccensis*) FOR XENOESTROGEN TREATMENT

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ABSTRACT

The incidence of water contamination by endocrine-disrupting chemicals (EDCs), xenoestrogen such as bisphenol-A (BPA) and estradiol (EST) is increasing in recent decades. This study was aimed to synthesize and characterize activate carbon derived from agarwood (agar AC) for xenoestrogen adsorption. The synthesis was conducted by physicochemical activation using sulfuric acid which was employed as an activator at 180 ^{o}C activation temperature followed by calcination under nitrogen condition. Both BPA and EST were detected in six selected rivers and lakes in Malaysia. Agar AC was characterized by electron microscopy, X-ray diffraction, micro-Raman, Fourier transform infrared spectroscopy, and Zeta potentials to determine which mechanism produced optimal result for the adsorption of BPA and EST. The carbon porosity was determined by nitrogen adsorption-desorption isotherm at 77K using Brunauer-Emmett-Teller (BET) method. The prepared agar AC was mainly micro and mesoporous in nature with BET surface area 1092 m^2/g. Optimization results showed an optimum removal of BPA was 86% (at carbon dosage 1wt.%, pH 7, contact time of 180 minutes and temperature of 50 °C) and EST removal was 79% (at pH 11, carbon dosage 4 wt.%). Experimental data were fitted into the Langmuir and Freundlich adsorption isotherm models. Both Langmuir and Freundlich isotherms predicted BPA adsorption of agar AC at R^2 values greater than 0.99, signifying chemisorption and physio-sorption processes. The Freundlich isotherm gives a better amount of EST uptake onto prepared activated carbon, indicating heterogeneous chemisorption process. In conclusion, the adsorption capacity of prepared agar AC showed high adsorption capacity (BPA 439 mg/g, EST 1664 mg/g) compared with the commercial AC. The implication of this study provides green adsorption with high sorption capacity material that can be applied for the removal of BPA and EST from water bodies such as rivers and lakes.
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SINTESIS DAN PENCIRIAN KARBON YANG TERAKTIFKAN DARIPADA GAHARU (*Aquilaria malaccensis)* UNTUK RAWATAN XENOESTROGEN

ABSTRAK

Kejadian pencemaran air oleh bahan kimia yang mengganggu endokrin (EDCs), xenoestrogen seperti bisphenol-A (BPA) dan estradiol (EST) telah meningkat dalam beberapa dekad ini. Kajian ini bertujuan untuk menghasilkan dan memperincikan karbon aktif yang diperoleh daripada gaharu (agar AC) bagi penjerapan xenoestrogen. Sintesis dijalankan dengan pengaktifan fizikokimia menggunakan asid sulfurik yang berfungsi sebagai bahan pengaktif pada suhu pengaktifan 180 ^{o}C diikuti dengan kalsinisasi di bawah keadaan nitrogen. Kedua-dua BPA dan EST dikenalpasti di dalam enam sungai dan tasik terpilih di Malaysia. Agar AC diperincikan dengan mikroskop elektron, pembelauan sinar-X, mikro-Raman, Fourier transform infrared spectroscopy dan potensi zeta untuk tentukan yang mana mekanisma yang mana dapat menghasilkan keputupen yang optimum BPA dan EST. Keliangan karbon ditentukan dari isotherm penjerapan-penyerapan nitrogen pada 77K menggunakan cara Brunauer-Emmett-Teller (BET). Agar AC yang disediakan secara umumnya mikro dan mesopore keadaannya dengan luas permukaan BET 1092 m^2/g. Hasil optimisasi menunjukkan penyingkiran optimum BPA adalah 86% (dos karbon 1wt.%, pH 7, 180 minit tempoh pendedahan dan suhu 50 °C) dan penyingkiran EST adalah 79% (pada pH 11, dos karbon 4 wt.%). Data eksperimen dimasukkan ke dalam model isotherm penjerapan Langmuir dan Freundlich. Kedua isotherm Langmuir dan Freundlich menjangkakan penjerapan BPA agar AC pada nilai R^2 lebih besar daripada 0.99, menunjukkan proses penyerapan secara kimia dan fizikal. Isotherm Freundlich memberikan amaun pengambilan EST yang lebih baik pada karbon aktif yang disediakan, menunjukkan proses penyerapan kimia secara heterogen. Kesimpulannya, penyerapan agar AC menunjukkan kapasiti penyerapan yang tinggi (BPA 439 mg/g, EST 1664 mg/g) dibandingkan dengan AC komersial. Implikasi kajian ini menyediakan penjerapan hijau dengan bahan kapasiti penyerapan yang tinggi yang boleh digunakan untuk penyingkiran BPA dan EST daripada sumber air seperti sungai dan tasik.

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LIST OF ABBREVIATIONS

PEXES	pharmaceutical estrogens and xenoestrogens
WWTP	wastewater treatment plant
STW	sewage treatment works
EE2	17α-Ethynylestradiol
E_2	17β-Estradiol
NP	4-Nonylphenol
4-T-BP	4-T-Butylphenol
OP	Text-Octylphenol-4
PAC	Prepared Activated Carbon
AC	Activated Carbon
AP	Alkylphenols
ADHD	Attention-Deficit/Hyperactivity Disorder
BE	Beryllium
BPA	Bisphenol A
BET	Brunauer, Emmett, and Teller
DEHP	Di-(2-Ethylhexyl) Phthalate
DBP	Dibuthyl Phthalate
DEP	Diethyl Phthalate
DES	Diethylstilbestrol
DMP	Dimethyl Phthalate
FESEM	Field Emission Scanning Electron Microscopy
EDCs	Endocrine-Disrupting Chemicals

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EDX	Energy Dispersive X-Ray Spectroscopy
EST	Estradiol
EA	Estrogen Activity
ER	Estrogen Receptor
ERs	Estrogen Receptors
ERE	Estrogen-Response Element
E1	Estrone
APE	Ethoxylates
FTIR	Fourier Transform Infrared Spectroscopy
CNTS	Carbon Nanotubes
GAC	Granular Activated Carbon
HCL	Hydrochloric Acid
H2O2	Hydrogen Peroxide
HNO3	Nitric Acid
NPES	Nonylphenol Ethoxy-Lates
OPES	Octylphenol Ethoxylates
OCPS	Organochlorine Pesticides
H3PO4	Phosphoric Acid
PAES	Phthalate Esters
PCBs	Polychlorinated Biphenyls
PCDDS	Polychlorodibenzo-P-Dioxins
PAHS	Polycyclic Aromatic Hydrocarbons
K2CO3	Potassium Carbonate
KOH	Potassium Hydroxide
K2HPO4	Potassium Phosphate Dibasic
PXR	Pregnane X Receptor
SHBG	Sex Hormone-Binding Globulin
NaOH	Sodium Hydroxide

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H2SO4	Sulfuric Acid
U	Uranium
XRD	X-Ray Diffraction
YES	Yeast Estrogen Screen
ZNCL2	Zinc Chloride
MO	Methyl Orange
EC	Electrical Conductivity Measurements
CHNOS	Total Carbon, Hydrogen, Nitrogen, Oxygen and Sulfur Content
CEC	Cation Exchange Capacity
AAS	Atomic Adsorption Spectrometry
WEOC	Water-Extractable Organic Carbon
OVOT	One Variable at One Time
CTAB	Cetyl Trimethyl Ammonium Bromide

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CHAPTER 1

INTRODUCTION

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1.1 Introduction

This chapter discusses the research background of the hormone xenoestrogen (Bisphenol A and Estradiol) for xenoestrogen treatment Application. Next, the water pollution treatment and preparations of activated carbon have are reported. The research problem statement, objectives, scope of the research are described at the end of this chapter.

Most pharmaceutical estrogens and xenoestrogens (PEXES) are introduced directly into surface waters through municipal wastewater treatment plant (WWTP) effluent sources, also called sewage treatment works (STW)(Daughton et al., 2010). They also stet that the low concentrations of individual pharmaceutical estrogen (possibly exceeding the catabolic enzyme affinities of sewage microbiota), coupled

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with their metabolic "novelty," (increase polarity) leads to incomplete removal from STWs. The focus on PEXEs has been on their interaction with the hormone receptors and the subsequent regulation of target genes. Following binding to the hormone receptor, PEXEs may either stimulate or inhibit gene transcription in a manner similar to the natural hormone or they may inactivate gene transcription by forming receptor-ligand complexes with conformations that are unfavorable for activation (EUR 1996). Some substances have however, been found to exert both agonistic and antagonistic effects on endocrine receptors (EUR 1996). Compounds having different mechanisms of action may cause similar biological changes. For instance, antagonists to the androgen receptor may give effects similar to those caused by estrogen receptor agonists. Besides interaction with hormone receptors, PEXEs may interfere with transport proteins, alter the synthesis and biotransformation of hormones, have direct toxic effects on the gonads or have adverse effects on the hypothalamus, the pituitary or endocrine glands(Wright-walters, 2009).

Municipal wastewaters are a complex mixture containing estrogens and estrogen mimics called xenoestrogens,(Kidd et al., 2007) natural and synthetic xenobiotics, household and agricultural chemicals, pharmaceuticals, hormones, and other compounds, many of which remain unidentified (Stevens, 2003). The majority of natural and pharmaceutical estrogens excreted by humans as well as xenoestrogens from numerous domestic and municipal sources (e.g., detergents, plastics, cosmetics,) enter WWTPs (Norris 2007). STWs receiving domestic and pharmaceutical waste release a complex (and ill-defined) mixture of natural and synthetic chemicals into the aquatic environment, due to their partial or complete resistance to biodegradation during the treatment process(Rodgers-Gray, Smith, Ashcroft, Isaac, & Dunn, 2004).

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Most of these compounds are retained in biosolids and a smaller portion typically appears in the wastewater effluent depending on the chemical and the type of treatment and retention times. Currently, more than half of the Biosolids produced by municipal wastewater treatment systems is applied to land as a soil conditioner or fertilizer and the remaining solids are incinerated or landfilled(King, Ballereau, Sch??rer, & Jobling, 2006). These disposal practices provide numerous routes for xenoestrogen reentry into environmental media and ultimately surface water. The use of biosolids as a soil conditioner and fertilizer allows for pharmaceutical estrogens and xenoestrogens exposure through the food supply chain and also reentry into surface water systems through run off, and contaminated groundwater outflow. Through incineration, compounds such as dioxins and furans are released into the air and may be deposited in watersheds through wet and dry deposition. Thus, there are many routes of reentry of xenoestrogens in and attached to the surface of biosolids from WWTPs possibly, increasing their environmental concentration and exposure routes for both humans and animals. There are over 16,000 municipal WWTPs nationwide and over 75% of the nation's population is being served by centralized wastewater collection and treatment systems. The remaining population uses septic or other onsite systems(Browning-Aiken, Richter, Goodrich, Strain, & Varady, 2004), which have not been adequately studied for xenoestrogens release. However, due to their high failure rate and lack of maintenance, could be considered potential non-point releasers of estrogenic compounds. Therefore, there can be an extremely varied mixture of pharmaceutical estrogens and xenoestrogens reentering surface waters possibly contaminating municipal drinking water supplies. But, what are the environmental concentrations of these compounds and are these concentrations significant enough to cause harm to human and wildlife health. Is human pharmaceutical estrogen and xenoestrogen

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exposure a valid public health concern. Activated carbon is widely used in environmental technology because it is effective for removing organic chemicals, heavy metals, unpleasant tastes from water and odors from gases (Jain, Ong, Jayaraman, Balasubramanian, & Srinivasan, 2016)[6 8]. However, high cost restricts wider application of activated carbon. So, researchers have been looking for low-cost precursors for activated carbon preparation. Many studies have investigated the preparation of activated carbons from waste materials or renewable sources such as stems, stalks(Girgis & Ishak, 1999) [10](Y. Sun et al., 2012), hulls (Gautam, Mudhoo, Lofrano, & Chattopadhyaya, 2014), oil-palm-shell(Arami-Niya, Daud, & Mjalli, 2011), Tamarind wood(Gautam et al., 2014)(Menon & Rao, 2012) and sugarcane bagasse(Ezebor, Khairuddean, Zuhairi, & Lim, 2014) to further decrease manufacturing cost and to solve environmental problem. Arundo donax Linn is extensively distributed in wetlands which have excellent performance in purifying wastewater (Y. Sun et al., 2012). Thus, abundant biomass of A. donax Linn is generated every year. The high content of carbon makes it a good raw material for activated carbon preparation. This study agree with four goals (3, 6, 14, and 15) of Sustainable development goals, as shown as in Figure 1.1.

Figure 1.1. The 17 Sustainable Development Goals

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1.2 Xenoestrogens

Xenoestrogens vary in their chemical structures, uses and properties. They interact with estrogen receptors or induce effects typically regarded as estrogen responsive or female-related. The main classes of xenoestrogens include hormones, surfactants/detergents, pesticides, and plastic-related compounds (e.g. bisphenol A and phthalates). A list of xenoestrogens and their relative potency to E2 shows there are several orders of dynamic range between the compounds. Only 17α-ethinylestradiol (EE2; the birth control pill hormone) is more potent than E2. The other natural estrogen hormones, estrone (E1) and estriol (E3), are less potent than E2 but more potent than other synthetic compounds. The estrogenicity of mixtures is attributable to the additive concentrations of the constituent Xenoestrogens adjusted by their relative potency to E2 (Thorpe et al., 2003),(Filby, Thorpe, Maack, & Tyler, 2007).as shown in Table 1.1.

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Table 1.1
Types of Xenoestrogens

Abbreviations/ acronyms	Definition
E1	Estrone
E2	17β-estradiol
E2-eq	E2 equivalent factor
EE2	17α-ethinyl estradiol
E3	Estriol

Human endocrine systems are known to control metabolic processes such as behavioural, growth, gut, cardiovascular, kidney, nutritional, and reproductive functions (World Health Organization, 2002). The main functions of endocrine systems

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are to facilitate the reaction of the targeted tissue towards a signal and to maintain the homeostasis in order to avoid major fluctuations. The integration of all endocrine systems happens through cross-talk. For instance, the reproductive function requires information related to age and nutritional status. However, it may have an impact to its surround up onto several endocrine systems which can be related to reproductive tissue, bone growth, and functions that related to cardiovascular. For certain endocrine systems, the homeostatic programming has been proven during the development of a fetal/neonatal to witness the disparity at this stage of life, which can be impacted from the environmental area (Owen, Andrews, & Mattews, 2013). It has been demonstrated in a recent report that the combination of wildlife, laboratory animals, and epidemiology data led to a suggestion of a larger role for endocrine-disrupting chemicals (EDCs) (Birnbaum, 2013). By taking into consideration of the human and animal data, the report will establish a stronger prospect whereby during the fetal life and/or puberty period, the EDCs exposure has a crucial role in the production of reproductive problems, asthma, infections, endocrine-related cancers, diabetes, and attention-deficit/hyperactivity disorder (ADHD) in both male and female (Birnbaum, 2013). Hormones are known to be the chemical messengers of the endocrine systems and they are free into the bloodstream by several glands and tissue, as shown in Figure 1.2.

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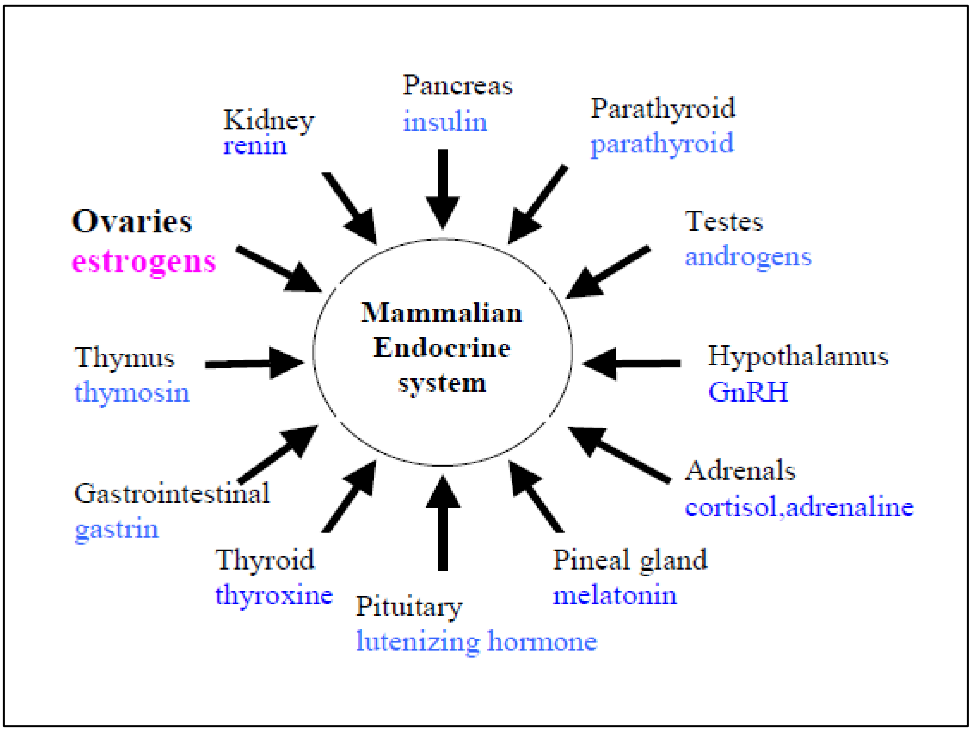

Figure 1.2. Endocrine System of Generating Glands and Tissues (Norris, 1996)

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Oestrogen is a type of hormone that encourages the establishment and maintenance as well as regulation of the female sex characteristics. Meanwhile, xenoestrogens imitate the impact of endogenous estrogen after an attachment to the estrogen receptors in the human body (Al-agha, Aiash, & Tatwany, 2015). Originally, the word ‘Xeno’ is based on the Greek word ‘Xeons’ which connotes ‘external’. Therefore, xenoestrogens are foreign compounds enter the body and partially imitate the estrogens’ actions. Generally, xenoestrogen is defined as an artificial estrogens that can be abundantly present in the environment, pesticides, plastic products, industrial wasteland, and in other daily-used products (Al-Agha et al., 2015). Additionally, Xenoestrogens can be synthetic such as plasticizer Bisphenol A (BPA) or naturally occurred compound such as genistein in soy. According to Massart, Harrell, Federico, and Saggese (2005), several chemicals in the environment contain estrogenic activity which referred to be as ‘xenoestrogen’ as they are synthesized outside the body. In 1938, synthesis of the

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diethylstilbestrol (Figure 1.3) was conducted by Dodds and Lawson. According to them, it is a chemical with strong estrogenic properties (Dodds & Lawson, 1938).

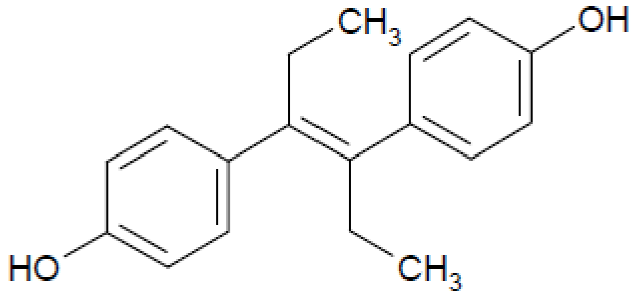

Figure 1.3. Chemical Structure of Diethylstilbestrol (Mueller, Hall, Swope, Pedersen, & Korach, 2003).

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1.3 Sources and Properties of Xenoestrogens

Endocrine Dirupting Chemicals (EDCs) are known to be a natural or synthetic compounds that hinders the function of the endocrine systems (Bidgoli, Eftekhari, & Sadeghipour, 2011). These naturally occurring chemicals or synthetic compounds are known as xenoestrogens. The common natural xenoestrogens are phytoestrogens and mycotoxins. Phytoestrogens can be found abundantly in several types of floras such as in tomatoes and oranges (naringenin), soybeans (genistein), yams (diosgenin), and grapes (resveratrol). Meanwhile, mycotoxins can be found abundantly in the mold of fusarium and fungi (Takemura et al., 2007). Due to extensively used or released during the process of industrial manufacturing, agricultural production process, and food packaging, there are abundante of synthetic Xenoestrogens in the environment. In agricultural production, chlorinated organic fungicide, herbicides, and insecticides

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remain to be the residues on dairy products, eggs, and meat (Lin et al., 2014). Zhang, Tao, Yuan, Liu, and Wang (2016) mentioned that plasticizers known to have xenoestrogen effects, especially polychlorinated biphenyls (PCBs), 4-nonylphenol (NP) and 4-text-octylphenol (OP), phthalate esters (PAEs), and BPA. Meanwhile, combustion by-products that include polycyclic aromatic hydrocarbons (PAHs), benzopyrene and dioxins are other kinds of xenoestrogens (Zhao Y, Chen X, Liu X, Ding Y, Gao R, Qiu Y, & Wang Y, 2014). In medical treatment, other synthetic pharmaceuticals are used such as diethylstilbestrol (DES), 17α-ethynylestradiol (EE2), selective estrogen receptor modulators (e.g., tamoxifen and raloxifene), and letrozole (Xu, Liu, Gu, Huang, & Pan, 2017).

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1.4 Environmental Fate

In drinking water, groundwater, and water surface, trace levels of xenoestrogens and their metabolites can be detected. The sewage from the wastewater treatment plants in the sediments, in the air, and on dust are discharged from aquaculture (Xu et al., 2017). It is mentioned that the xenoestrogens and estrogens can be discovered in the receiving water such as outfalls, upstreams, and downstream from the river sites. It is discovered that from these waters, the presence of xenoestrogens is much higher in concentration compared to the estrogens (Ying, Kookana, Kumar, & Mortimer, 2009). In the wastewater treatment plants, the ruminants of the wastes comprised of natural estrogens 17β-estradiol (E2), estrone (E1) and the synthetic estrogen 17α-ethynylestradiol (EE2) are utilized widely as compounds which are responsible for the estrogenic activity in the oral contraceptives (Ying et al., 2009). In medical and veterinary therapy, drugs that

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have been used are reported to excrete in the human's faces and urine and widely detected in the environment.

Additionally, other synthetic xenoestrogens can diffuse into the environment when disposed to the atmosphere and through soil erosion. Unfortunately, contemporary wastewater treatment systems cannot totally eliminate xenoestrogens (Xu et al., 2017). Thus, they will mixed with the natural waters and accumulate within the aquatic organisms. Meanwhile, human exposure can happen when a person consumed the water or any aquatic organisms such as amphibians and fish even at 1.0 µg/L concentration (Z. Xu et al., 2017). These exposures to xenoestrogens subsequently may lead to numerous health problem in human.

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1.5 Effects of Xenoestrogens on Organisms

That the most predominant xenoestrogen compounds which exhibit disrupting impacts on both human and animal endocrine systems are reported alkylphenols (AP) ,(Gurban et al. 2015). This includes BPA, polybrominated biphenyls organochlorine herbicides and pesticides, commercial formulations of Ethoxylates (APEs) comprise of isomers, homologs, and oligomers. However, as commercially used today, non-ylphenol ethoxy-lates (NPEs) group is more commonly known as it represents 80% of the world market, while another 20% of the world market is represented by the octylphenol ethoxylates (OPEs) (Gurban et al., 2015).

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1.5.1 Impact of Xenoestrogen to Human Health

EDCs disturb the homeostasis and normal endocrine function in humans as it has an adverse impact on the show of various physical behavior at even low exposure levels (Xu et al, 2017). Among the main effect of these xenoestrogen that received great attentions are cancer, impaired reproductive function, neuro-behavioral and immune function.

Generally, the development and the function of the reproductive organs are affected by the estrogen. This is because estrogen encourages the production of epithelial cells which is present in the female's mammary gland and in the male's prostate (Kushner et al., 2003). (Rignell Hydbon et al. 2005) investigated the integrity of human sperm chromatin on exposure to PCBs and dichlorodiphenyl dichloroethylene. The study was conducted on 176 fishermen in Sweden with high and low consumption of fatty fish (a significant source of endocrine disruptor). Results showed that xenoestrogens have a negative effect to the reproduction system such as decreasing the number of human sperm (change in sperm chromatin structure), deficiency in infertility, increase the number of impulsive abortions, and drop in the ratio of babies' male as well as abnormalities of the male reproductive system. It was claimed that the exposure to the organochlorine pollutants such as PCBs may lead to negative consequences on the human's sperm chromatin integrity (Rignell-Hydbom et al., 2005). In other parts of the world, PCBs and pesticides are noted to be the cause for the reduction of fertility. In a study of analyzing the blood serum from 2000 Americans and 50 Southeast Asian women, the result revealed that there is a connection of PCBs

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and DDE levels which can affect the human menstrual cycles (including cycle length, regularity, and bleeding duration).

Eventually, it would affect other endpoints such as fertility, pregnancy, and reproductive cancers as the adverse influence of endocrine disruptor on public health (Cooper, Klebanoff, Promislow, Brock, & Longnecker, 2005). Besides, it has been reported that mentioned POP exposure (PCBs concentration range; 50-200 ng/g lipid; *p,p'*-DDE concentration range: 250-1500 ng/g lipid) too can alter the amount of ejaculated Y-bearing spermatozoa in the human populations and significantly influenced the Y:X chromosome ratios. This depends on the degree of exposure and doses which can cause the varying of ratio in the Y and X chromosome (Ludwicki et al., 2006). In Human Studies by Buck et al. (1999), it is mentioned that there is a probable correlation between maternal exposure to the contaminated Great Lakes fish and a reduction in fertility. Xu et al. (2017) also mentioned that women who are exposed to a range of pesticides may also experience an increase in the number of impulsive abortions. The risk is high specifically for women who working in the agricultural sector (Xu *et al*., 2017).

1.5.2 Wildlife Effects

The risks of xenoestrogens have attracted the interest of many scholars globally to conduct studies related to fishes and rats. This is because when xenoestrogens are being exposed to various substances and doses with different animal models, it may have negative effects. Metabolism of pesticides is a causative factor in various instances of

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reproductive anomalies in amphibians and birds. An example is the account of alligators in Florida's Lake Apopka that showed the altered sexual variation of the male reproductive tract, apparently resulting from the spill of pesticide into the lake (Singleton & Khan, 2003). Certain xenoestrogens are prompt to biphasic dose and tend to react with some animal models. Generally, xenoestrogens have inhibitory or toxic effects at higher concentrations and they also stimulate estrogenic impacts when they are at low exposures (Xu et al., 2017). For instance, when the concentration of xenoestrogens is low, it will cause a negative impact on the sperm's parameters while at when the concentration is high, it may either have the contrasting effect or no changes (Zateeka et al., 2014). Previous literature revealed that the xenoestrogens may develop reproductive disorders at sexual differentiation. Furthermore, they can also interrupt the gene expression as well as the establishment and the role of the reproductive organs during the early-life exposure (Liu et al., 2014). According to Corcoran et al. (2014), persistent antifungal agent clotrimazole is commonly known to be found in the aquatic environment and it comprises of various tissues of *Cyprinus carpio* which can be the regulator of hepatic detoxification of gene and pregnane X receptor (PXR). Thus, exposure to xenoestrogens for a long period of time can affect in many ways such as the development of gonad, the success of one's fertility, synthesis and viability of germ cells, and sex differentiation and gender ratios. This will eventually lead to the depletion of community, changes in the species composition, or even extinction of species (Huang et al., 2015).

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1.5.3 Beneficial Effects of Xenoestrogen

Xenoestrogens such as phytoestrogens are the naturally occurring compounds which are commonly found in food which has certain functions such as nutritional supplements and as a replacement to the hormone auxiliary treatment due to the health benefits they have (COT, 2013).

- Diseases related to cardiovascular
- Hormone dependent cancers, especially breast cancer
- Cholesterol related disorder
- Postmenopausal conditions such as osteoporosis
- Stomach disorder.

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1.6 Water Pollution

Human activities that lead to increasing disposal of materials into the aquatic environment, water resources are said have polluted where by the biological and chemical have changed the water characteristics to the extent that its usefulness for various purposes is evidently devalued. In this study, the quality of the physicochemical and biological traits of the water serves as an indicator to provide greater insights and solid proof of the situation prevalent to the trophic status of the water bodies. Countries all over the world are concerned with the impacts caused by polluted drinking water as there can lead to presence of water-borne diseases, which are the main source of mortality and morbidity. Thus, supply of clean drinking water is crucial for overall human health as well as for the development, growth, and survival of a children population (Anderson et al., 2010). According to the World Health Organization's

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report in 2005, about 1.8 million people are suffering from diarrheal disease in a year. Individuals with compromised immune system, specifically those with AIDS, are more susceptible to water-borne infections (Anderson et al., 2010). Afroz et al. (2014) summarized the significant factors of water pollution, as shown in Figure 1.4.

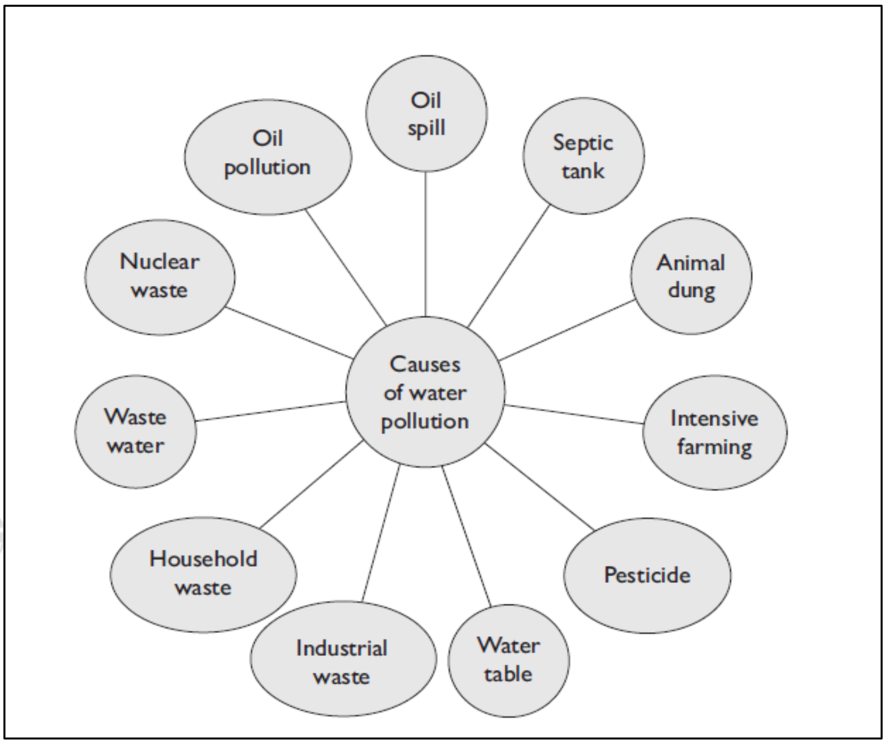

Figure 1.4. Major Causes of Water Pollution(Afroz & Masud, 2015).

1.7 Treatment of Xenoestrogens -Bearing Wastewater

The human population is rapidly and continuously growing and urbanizing. This can be a problem in securing the water resources as the habit of disposing into wastewater will progressively becoming more challenging (Angelakis & Snyder, 2015). Using the collection sewers, the wastewater is transported to the centralized wastewater treatment plant. In addition, the cost of keeping and transferring domestic water is said to be less

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economically viable. Advance technologies assist in lowering human energy and enhance the dependability as emerging contaminants are problematic to be explained in general. Angelakis and Snyder (2015) mentioned that the history and the current occurred disease spread via water have caught the public's concern regarding the usage of reused water. Online sensors, membranes, and advanced oxidation are among the advanced technologies that can facilitate to ease the perception of the public. Further insight into how the reused water is engineered compared to the existing sources is needed to be persuasive. Due to the concern of mixture toxicity, it has gained greater challenges to the emerging chemical constituents. This is because certain mixtures contain an endless number of calculations. Thus, there is a quick biological screening assay which gained the attention as a mean for rapid and systematic evaluation of the chemicals in the complex mixture in water (Angelakis & Snyder, 2015). It was mentioned that high-throughput bioassay is being used successfully for identifying the presence of chemicals qualitatively and quantitatively in a wide collection of biological endpoints related to public health. These could be expensive but safer when compared to conventional methods like membrane filtration, chemical precipitation, and sulfide precipitation. The most efficient method of treatment especially using adsorbent is adsorption. In the adsorption process, pollutant ions or molecules migrate from solution to the surface of the adsorbent. Adsorbents are classified as commercial or engineered sorbent based on the mode of availability, synthesis or processing. Activated carbons (both powdered activated carbon (PAC) and granular activated carbon (GAC)), carbon nanotubes and natural adsorbents (biochar, agro-waste, clay, etc) are common adsorbents used for the treatment of pollutants in water. However, the adsorption process efficiency depends mainly on the chemical and physical features of the adsorbent such as surface functional groups, ash content and pore structure. Due to the

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high cost of commercial adsorbents, development of low cost and environmentally friendly adsorbent from agricultural waste source is the very interesting and become focus of the current research. This study aims to establish the preparation of activated carbon from agarwood (*Aquilaria malaccensis*) for xenoestrogen uptake from aqueous solution.

1.8 Preparation of Activated Carbon as Adsorbent

The activated carbon mostly used as an adsorbent for wastewater and water treatment. The distinction of high surface and well-internal developed pore structure, random amorphous structure with activated carbon (AC) is being used extensively due to the effectiveness of the adsorbent to eradicate the wide-ranging pollutants from the aqueous solution (Pio et al., 2015). AC is prepared from carbonaceous materials such as coconut shell, peat, wood, bones, palm kernel shell, rice husk, petroleum coke, and coals, using physical (gas) activation, chemical activation or both methods (Yahya et al., 2015).

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1.8.1 Physical Activation

During the physical activation under an inert atmosphere, the starting material is initially being carbonized and the biochar is submitted to part of gaseous steam, air/oxygen, carbon dioxide (CO_2), or a mixture of both (Bedia et al., 2018). In the first step, carbonization is the precursor which altered into a structure of fixed carbon with the rudimentary pore. The carbon produced is proportional to the biomass composition. This is because the carbonization eliminates generally non-carbon components such as

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the heteroatoms at low temperature. Secondly, the gasification procedure will select to remove the carbon atoms which are reactive by producing the porosity in order to activate the final carbon structure. In order to activate the CO_2 or H_2O, these substances widen the absorbency of the carbon which resulted in activation of carbon with wider porosity at high burn off values. The following formula shows the burn-off mass remove during the gasification:

$$\text{Burn off } (\%) = m_g/m_o.\ 100 \qquad (1.1)$$

Where m_o represents biochar mass, and m_g denotes the leftover mass of activated carbon. Normally, C is used to express the burn-off. A burn-off value that is low brings about high carbon yields and low in porosity. On the other hand, a burn-off value that is high develops activated carbons with well-established porous texture, but with low carbon yields. The limitation of the utmost effect on the characteristics of the got activated carbon is the temperature of gasification. Narrow porosity is obtained when the reaction of gasification is controlled even if the temperatures were low. However, when the temperatures of gasification are high, wider pores are developed and the occurrence continues under the diffusion control (Bedia et al., 2018). As shown below, the main gasification reactions involve H_2O, CO_2, and O_2 as the oxidizing reagents. The reaction of the gasification is labelled to be intensely exothermic due to the strict control of the reaction (van Oss, 1990).

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$$C + H_2O \rightarrow CO + H_2 \quad \Delta H_{25}°C = +117\ kJ.mol^{-1} \qquad (1.2)$$

$$C + CO_2 \rightarrow 2CO \quad \Delta H_{25}°C = +159\ kJ.mol^{-1} \qquad (1.3)$$

$$C + O_2 \rightarrow CO_2 \quad \Delta H_{25}°C = +386\ kJ.mol^{-1} \qquad (1.4)$$

$$2C + O_2 \rightarrow 2CO \quad \Delta H_{25}°C = +\ 256\ kJ.mol^{-1} \qquad (1.5)$$

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1.8.2 Chemical Activation

There are three main steps in the preparation of the precursor. The steps involve dehydration, carbonization, and activation. In the dehydration process, chemical activation starts with the mixing of the activating agent with the precursor. The chemical agents comprise of various salts, and acids or bases such as sulphuric acid (H_2SO_4), phosphoric acid (H_3PO_4), sodium hydroxide (NaOH), iron(III) chloride ($FeCl_3$) and potassium hydroxide (KOH), (Iriarite-Velasco et al., 2016). The activating agents are the dehydrating agents and they inhibit with the creation of tars, volatiles, and any products unwanted that can be produced during the phase of carbonization. In the step of carbonization, the biochar has highly significant fixed carbon content which later joined the activating agent. Initially, the starting of the material-activating agent mixture is treated to the thermal temperature ranging from 400 °C to 1000 °C. The final step requires the carbonized solid to be washed and removed from the pores. During the final activated carbon phase, the remaining products of the reaction were released to the final porous structure. The activating agents are the main variables to affect the structure of the final porous. Furthermore, the ratio of the mass between the chemical activating agents and precursor can be obtained. Generally, NaOH and KOH are strong bases which generate high large surface area microporous carbons (Fernandez-Ruiz et al., 2018). Meanwhile, the porosity is tailor-made with other activating agents due to the activating temperature and mass ratio. Therefore, it can be concluded that the higher porous development is due to the higher activation temperature. Furthermore, a low mass ratio commonly results in microporous carbons. Meanwhile, high activating ratio widens the porosity which eventually promotes the creation of macrospores and mesoporous (Bedia et al., 2018). The chemical activation process involves single step

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treatment, greater yielding, shorter in time, and less activation temperature (Yahaya et al., 2015; Fernandez-Ruiz et al., 2018). The current study presents a design to determine the amount of xenoestrogen in the study area and the type of xenoestrogen and the water treatment by using activated carbon from the agarwood. To our knowledge, up to now no determination of the amount of xenoestrogen in water and treatment of xenoestrogen in water by using activated carbon from the agarwood has been reported. Thus, with manganese dioxide, the screen-printed carbon electrodes were altered. In order to show the properties of redox for oxidative phenols decomposition, room temperature in water solution was set for the preparation. The main activated carbon attains decent removal capacity for all compounds that had been tested without using another modifier. Therefore, it leads to the development of affordable adsorbent for the removal of xenoestrogens. This chapter presents greater insight on the knowledge of removing the xenoestrogens, the synthesis of activated carbons, factors that affect adsorption, and adsorption isotherms modelling for xenoestrogen treatment.

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1.9 Problem Statement

Humans are exposed daily to xenoestrogens in food (e.g., phytoestrogens, various pesticides) and from contact with detergents [e.g., nonylphenols (NP)] and ingestion of plastic additives from plastic bottles, metal beverage can linings and food packaging (e.g., phthalates, bisphenol A (BPA). In addition, many personal care products (e.g., shampoos, cosmetics, aftershave lotions) contain xenoestrogens such as phthalates, NP and BPA. Most of these pharmaceutical and xenoestrogens are introduced into the environment via municipal WWTPs. Treated WWTP effluents are directly discharged

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in rivers and lakes. A recent publication by the U.S. Geological Survey reported that reproductive hormones and estrogenic alkylphenols were present in 40% and 70%, respectively, of the surveyed U.S. surface waters (USEPA 2001). Thus, as rivers and lakes are used for municipal water sources, to help produce our food supply and for recreation, and as wastewater effluent water reuse increases, the presence and concentration of xenoestrogens in surface water becomes a valid public health concern. Advances in civilization coupled with rising population levels have resulted in an increasing need to treat and recycle available water resources. In the United States surface water provides for 62% of the public water drinking supply. Since 1950, irrigation has accounted for about 65% of total water withdrawals, excluding those for thermoelectric power. Historically, more surface water than ground water has been used for irrigation. Following use, water is returned to the aquatic environment, usually via STWs of varying processes and performance, which improves its quality, but it has a high probability of being withdrawn downstream for municipal or industrial reuse. In US cities with a high population density, the volume of effluent discharged from STWs can be considerable, sometimes contributing up to 50% of the flow of a river, a figure that can rise as high as 90% in periods of low rainfall. The challenges posed by EDCs such as BPA and estradiol (EST) in surface waters and sources of drinking water cannot be overemphasized (Verlicchi et al., 2010). The occurrence of BPA and EST in water even at low concentration accumulates over a long period of time may damage human health (Rosal et al., 2010). These substances are known to be persistent in the environment without the hopes of being degraded naturally. A major concern has been the possibilities of being passed into various potable municipal water systems. More so, a large number of EDCs are available in surface waters due to the discharge of wastewater from industrial processes, municipal conventional secondary treated

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effluents (such as; cosmetics, medicines, and general cleaning chemicals), stabilization lagoons and septic tanks leakages, and farming irrigation runoff (containing; pesticides and herbicides) (Guedidi et al., 2013).

There has been an alarming awareness of unintentional existence of pharmaceuticals and personal care products in numerous compartments of the aquatic environment. These are at a concentration capable of causing severe effects on the aquatic organisms (Ebele et al., 2017). This has raised concern due to increasingly extensive applications of pharmaceuticals and personal care products in human and veterinary medicine, resulting in the unending release of endocrine-disrupting compounds (EDCs) to the environment (Nikolaou et al., 2007). These EDCs, xenoestrogen including BPA and EST present in water bodies at a concentration capable of inflicting negative responses by interfering in the reproduction and growth of aquatic organisms (Evgenidou et al., 2015). A similar effect can be found in human consuming such fish and drinking water via bioaccumulation. Hence, the need to continuously monitor water source for this emerging contaminant and proper treatment of industrial effluents. This study focused on ameliorating the presence of EDCs in the environment, through the application of batch adsorption and kinetic study of BPA and EST in an aqueous environment. Researchers have adopted earlier types of methods and techniques such as ion exchange, electrochemical, and chemical precipitation have been faced with numerous challenges such as low efficiency, high cost of operation, these all because of secondary pollution during treatment processes. Conversely, the process of adsorption by using natural materials is the way forward in the treatment of pollutants such as BPA and EST on a sustainability scale. The study utilized activated agarwood as a sustainable material to adsorb BPA and EST in an aqueous environment.

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The reports are useful in the development of low-cost technology in the removal of toxic EDCs from contaminated water using agricultural by-products as adsorbents. More so, agarwood activated carbon (agar AC) as adsorbent serves as a form of waste management strategy and brings about the balance of materials in the ecosystem through utilization. It is worthy to note that the amount of agricultural waste generated in Malaysia has been on a steady increase over the years (Jumasiah et al., 2005). Agar AC is very beneficial to the nation of Malaysia, since it, naturally having an abundance of agarwood, hence the need to adopt agar AC towards adsorption of BPA and EST in the aqueous environment. These will contribute to the sustainability of the surrounding environment of the nation as a whole and on a global scale in general.

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1.10 Objectives of Research

The objective of this study is to synthesize activated carbon from agarwood to xenoestrogens treatment; consist of high surface area, high pore volume and great functional group and mechanical stability activated carbon as the adsorbent material. The synthesized activated carbon is then tested in Bisphenol-A and Estradiol adsorption from the water. To achieve this goal, five objectives should be accomplished

I. To monitor the water quality of the study area and determine the type and the concentration of xenoestrogen.

II. To synthesize activated carbon-based derived from waste agarwood (*Aquilaria malaccensis*).

III. To characterize the activated carbon of agarwood.

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IV. To optimize the agarwood activated carbon activity in adsorption of xenoestrogen from contaminated water.

V. To compare the performance and efficiency of the prepared agarwood activated carbon with commercial activated carbon in terms of their ability to adsorb the xenoestrogen from contaminated water samples.

1.11 Limitation of Study

Exposure of xenoestrogen to water it may affect the biological life because it is act as false messengers and disrupt the process of reproduction(Williams, Lech, and Buhler 1998). This research will determine the amount of xenoestrogen on the study area and the type of xenoestrogen and the water treatment by using activated carbon from the agarwood. The research will be into two parts: first part will be the monitoring the study area and identify the xenoestrogen type. The other part is the treatment of the contaminated water by using activated carbon. In this research will study the effect of the pH, the amount of activated carbon and the effect of temperature on the adsorption of xenoestrogen.

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1.12 Scope of Work

Exposure to xenoestrogens especially through water affects biological life due to the interference with homeostasis and normal endocrine function which adversely affect the performance of several physiological functionally. This research will determine the amount of xenoestrogen in the study area and the type of xenoestrogen and the water

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treatment by using activated carbon from the agarwood. The research will be into two parts: the first part will monitor the study area and identify the xenoestrogen type. In the second part, the prepared activated carbon is to be characterized by Field Emission Scanning Electron Microscope (FESEM), Brunauer-Emmet-Teller (BET), XRD, Fourier Transform Infrared Analysis (FTIR) spectra, Raman and Zeta potential. This was followed by xenoestrogens adsorption onto the activated carbon produced from the agarwood by chemical activation using H_2SO_4 as a function of adsorbent dosage, initial concentration, pH, and effect of temperature. Isotherms evaluation of the adsorption process was based on the adsorption data collected from the adsorption experiment batch.

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1.13 Significance of Study

This research is using an accurate method to identify and quantify the amount xenoestrogen in the study area and it may help to answer the above questions. The outcome of this study will provide a good data in the identification of the most dangerous hormone in the water to the human health and the animals because xenoestrogen is one of the factor led to the cancer. The identification of xenoestrogen types, and the concentration at the study area environments can effectively contributes to reducing the probability of the disease caused by xenoestrogen. In this work the activated carbon will be use from the waste of agarwood.

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