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CHEMICAL CATALYTIC AND BIOCATALYTIC PROCESS OF CLOVE OIL DERIVATIVES REVIEW

TURUNAN DARI MINYAK CENGKEH DENGAN PROSES KATALIS DAN BIOKATALIS (TELAAH)

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ABSTRACT

In 2011, Indonesian clove oil supply reached about 75 % (4,500 of 6,000 tons) of the world market. Utilization of clove oil and clove oil derivatives in aromatic chemical industry primarily as a mixture or additive of fragrances in the daily consumed product, such as perfumes, skin care products, deodorant, soap, shampoo, detergent, besides it is also used as an ingredient in the production of synthetic vanilla. The content of eugenol as the main compound in the essential oil in the clove flower, flower stalk and leaf have a range of 90-95 %, 83-95 % and 82-87 % respectively. The compounds content in clove oil is divided into two categories, phenolics (eugenol) and non-phenolic (beta-caryophyllene) that can be derivatized with various chemocatalytic and biocatalytic processes. Separation of the compounds in clove oil can be conducted by adding NaOH with repeated distillation. This process produces two layers product, the first layer contains eugenol and NaOH, while the second layer contains beta-caryophyllene. Derivatization of eugenol are conducted to produce various products such as vanilla, eugenyl ether, methyl ether eugenyl, eugenyl ethyl ether, eugenyl acetate, eugenyl cinnamate, dimmer eugenol and eugenyl benzoate, whereas derivatization of beta-caryophyllene are conducted to produce products such as caryophyllene oxide, kobusan, glycols, alcohols caryophyllene, β-caryolanylformate and kovanilformate, klovanildiformate, caryophyllene ketol. Biocatalysis or biotransformation can be defined as the use of biological systems (intact cells, cell extracts or isolated enzymes) to catalyze the conversion of a compound into another. Besides the common chemocatalysis system for derivatization of clove oil and clove oil compound such as eugenol into other compounds some biocatalysis systems were also described in this paper.

Keywords: Clove oil, eugenol, beta-caryophyllene, derivatization, chemical catalysis, biocatalysis

ABSTRAK

Pasokan minyak cengkeh Indonesia ke pasar dunia pada tahun 2011 mencapai sekitar 75 % (4.500 dari 6.000 ton). Pemanfaatan minyak cengkeh,dan turunan minyak cengkeh dalam idustri kimia aromatik terutama sebagai campuran atau aditif pewangi dalam produk yang dikonsumsi sehari-hari seperti parfum, produk perawatan kulit, deodoran, sabun, shampoo, deterjen, selain itu juga merupakan bahan antara dalam produksi vanili sintetis. Kandungan eugenol sebagai kandungan senyawa utama dalam minyak esensial dalam bunga, tangkai bunga dan daun cengkeh memiliki kisaran 90-95 %, 83-95 %, dan 82-87 %. Kandungan senyawa minyak cengkeh dibagi menjadi dua kategori, fenolat (eugenol) dan non-fenolat (beta-caryophyllene) yang dapat diderivatisasi dengan berbagai proses katalis kimia dan biokatalisis. Pemisahan kandungan senyawa minyak cengkeh dapat dilakukan dengan menambahkan NaOH dengan distilasi berulang. Proses ini menghasilkan produk dengan dua lapisan, pertama mengandung eugenol dan NaOH, sedangkan lapisan kedua mengandung beta-caryophyllene. Derivatisasi eugenol digunakan untuk menghasilkan produk seperti vanili, eugenil eter, metil eter eugenil, eugenil etil eter, eugenil asetat, eugenil sinamate, dimer eugenol dan eugenil benzoat. Sedangkan derivatisasi beta-caryophyllene, untuk menghasilkan produk seperti oksida caryophyllene, kobusan, glikol, caryophyllene alkohol, β-caryolanilformat dan kovanilformat, klovanildiformat, caryophyllene ketol. Derivatisasi bisa dilakukan dengan menggunakan proses kemokatalitik dan biokatalitik.

Makalah ini meninjau beberapa sistem kemokatalisis dan biokatalisis untuk derivatisasi minyak cengkeh dan komponen minyak cengkeh seperti eugenol menjadi senyawa lain. Biokatalisis atau biotransformasi dapat didefinisikan sebagai penggunaan sistem biologis (sel utuh, ekstrak seluler atau enzim terisolasi) untuk mengkatalisis konversi suatu senyawa lainnya. Selain sistem kemo katalisis yang umum digunakan untuk derivatisasi minyak cengkeh dan senyawa minyak cengkeh seperti eugenol menjadi senyawa lain, dalam makalah ini beberapa sistem biokatalisis juga dijelaskan dalam makalah ini.

Kata Kunci: *Minyak cengkeh, eugenol, beta-kariofilen, derivatisasi, katalisis kimia, biokatalisis*

INTRODUCTION

Clove oil

Indonesia is a very potential country for the development of the essential oil industry. *In 2011, Indonesian clove oil supply, reached 75 % (4,500 from 6,000 ton) of the world market*[1]. Although the clove oil has also been developed for the medical and cosmetics field, however the general public recognized clove only as a spice for cooking and ingredient in cigarettes[2].

Clove oil is an essential oil obtained from cloves (*Eugenia aromatica* or also known as *Syzygium aromaticum*) that belongs to the family of Myrtaceae[3]. Eugenol is the major component in the essential oils from the clove flowers, flower stalks and leaves which the content have range 90-95 %, 83-95 %, and 82-87 % respectively[4].

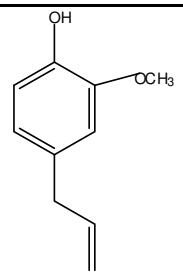
The oil yield ranged between 2-12 %, depending on the type and circumstances of raw materials, materials handling, as well as the manner and conditions of distillation[5]. Constituents of clove oil can be divided into two groups. The first group is a phenolic compound which eugenol is the major component. The second group contains compounds that are non-phenolic such as β-caryophyllene, α-kubeben, α-kopaen, hulumen, δ-kadien, and 1,3,5-trien Kadina[6].

Analysis of the components in clove oil (density at 30 ^{o}C = 0.9994[7]) using gas chromatographic showed two major peaks, eugenol (71.5 %) and β-caryophyllene (28.0 %).

Eugenol

Eugenol is a compound from the class of oxygenated hydrocarbons with the molecular formula is $C_{10}H_{12}O_2$. Chemical nomenclature of eugenol includes 4-allyl-2-methoxy-phenol, 1-hydroxy-2-methoxy-4-allyl benzene or 4-allyl guaiacol that are not optical[3]. Eugenol, is volatile, colorless or slightly yellow color and has a bitter taste. Eugenol may turn brown if in contact with air due to oxidation event[8]. Eugenol is soluble in alcohol, chloroform and ether, and sparingly soluble in water. Eugenol has a molecular weight of 164.20 g/mol[5]. Physico-chemical properties of eugenol based on Leody standard 1970[9] and based on EOA standards for trade[10], are presented in Table 1. Eugenol is a phenolic compound containing at least one hydroxyl group and more are forming ethers, esters or glycosides rather than independent compounds. The solubility of an ester or ether of phenol compounds in water is lower than the phenols compounds, while glycoside compounds are more soluble in water[11]. In a state of pure compound, simple phenol is a colorless solid, but usually oxidized and has dark color if it reacts with air. Water solubility increases with the increasing number of hydroxyl groups, but the solubility in polar organic solvents are generally high. Phenol has a small solubility in water, however it soluble in sodium hydroxide solution[11].

Table 1. Eugenol Physico Chemical Properties



Eugenol

Characteristics	Value [9]	Value [10]
Specific gravity (25/25 °C)	1.053 to 1.064	from 1.064 to 1.0702
Refraction Index (20 °C)	1.5380 to 1.5420	1.540 to 1.542
Purity (GLC)	-	Eugenol, min. 99 %
Sightings and color	-	to light yellow clear liquid
As the scent	-	of clove aroma
Solubility in 50 % ethanol	1:5 or 1:6	1:2
Optics round	-1°30'	

Source: Essential Oil Association, 1970[9] , PT. Aroma Indesso Indonesia, 2006[10]

RESULTS AND DISCUSSION

Derivatization of Eugenol by Chemical Catalysis

Dimer Eugenol

Focus will be on synthesis of dimer eugenol that can serve as an anti-oxidant and anti-inflammatory drug. The dimerization of eugenol is an oxidative phenolic reaction that can be carried out with solid acid or metal complex catalyst. These nanocatalysts are characterized as heterogenous catalyst and have advantages in the separation stage of the product which will be very useful for future industrial process. It has been reported that the dimer of eugenol, dehydrodieugenol has known to have less cytotoxicity and greater anti inflammatory activity than parent eugenols[12-15].

A famous scientist that have developed this particular field in the industrial market is the 2001 Nobel laureate, Ryoji Noyori, for his discovery of an asymmetric catalyst - chiral BINAP-rhodium complex which is currently used by Takasago Corporation for commercial industrial synthesis of menthol[16,17]. With his catalyst, stereoselective menthol is produced and side-products are eliminated. The reaction is selective hydrogenation.

Oxidative phenolic reaction using nanocatalysts can undergo in many pathways and produce a mixture of products. With the application of highly selective heterogeneous nanocatalysts, the reaction can be control for a specific pathway and eliminate the mixture of products. Heterogeneous catalysts are also easily separated from the product. Reaction pathways of oxidative phenolic reactions is displayed in Figure 1.

Dimerization involves the attack of a phenolic radical. The radical formed will undergo resonance and combine with other radicals in a coupling reaction. In this stage the stability of the catalyst is needed for the efficiency of reaction. The reaction can take place with an acid or metal complex catalyst which is able to accept electrons from the phenolic compounds[17,19,20] .

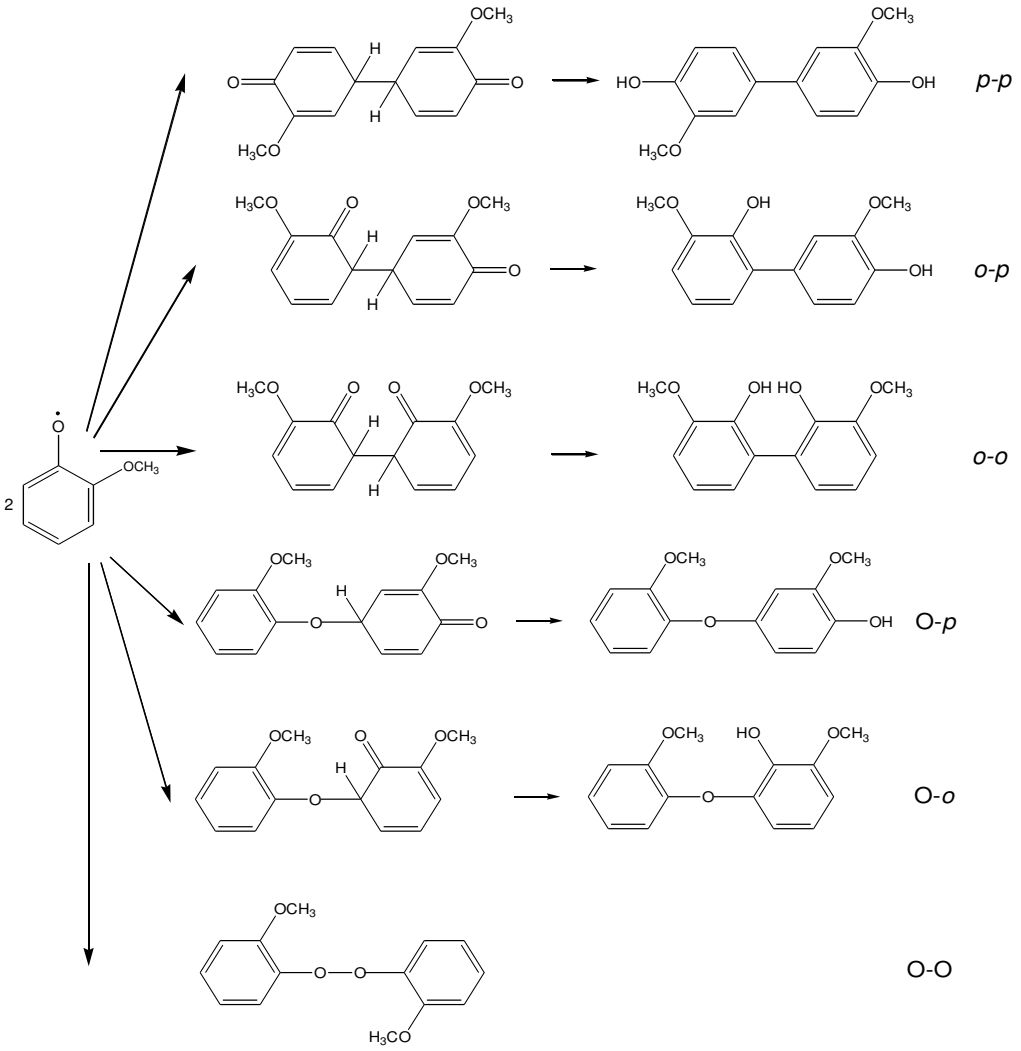


Figure 1. Reaction pathways of oxidative phenolic reactions[18]

Heterogeneous nano-catalysts prepared will be a solid metal-complex and solid acid catalyst. A solid metal-complex is inorganic–organic hybrids have been gaining much attention from the viewpoint of functional materials. 1,2 Host–guest interactions between inorganic layer compounds and organic compounds give a great potential to construct novel nano-structural materials[21]. Among the inorganic layer compounds, the clay minerals have prosperous properties such as facile intercalation of polar guest molecules and cations, reversible lamination and delamination, and swellability[22,23]. The metal-complex catalyst by the intercalation of organometal-complex into clay as summarized in Figure 2.

The concentration of hydrogen or acid can increase indefinitely since water mixes in all proportions with acids. Besides, with suitable equipment, we can prepare 'pure' acids with no water in them – most likely in conditions that are not standard. Acids whose pH < 0 are called super acids (Cotton, Wilkinson, & Gaus (1987: 221)[24]. Super acids are used to provide protons, and are believed even to force substances to accept protons. Similarly, there are super bases.

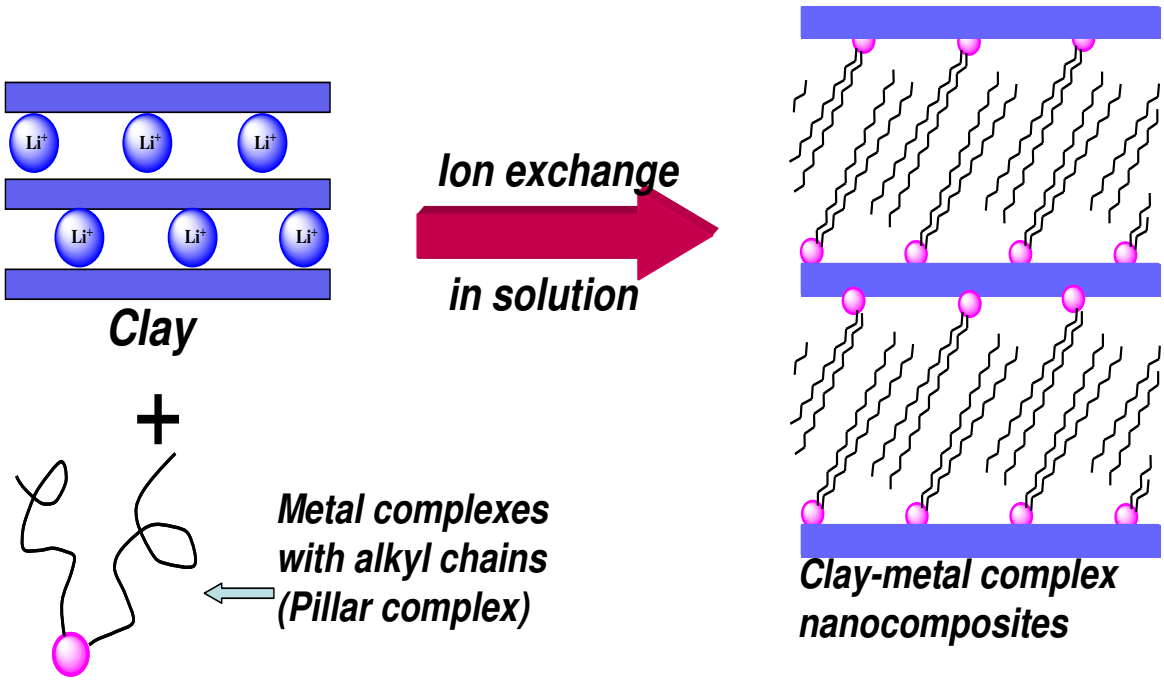


Figure 2. Intercalation of metal complexes into clay[21]

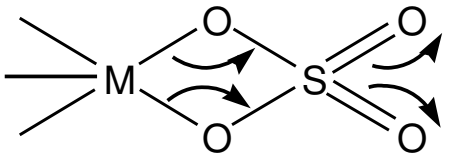
According to Cotton, Wilkinson, & Gaus (1987: 221)[24], super acids are necessarily non aqueous (i.e., have little or no water) since the acidity of any aqueous system is limited by the fact that the strongest acid that can exist in the presence of water is H_3O^+. In that system, a stronger acid than H_3O^+ loses its proton to H_2O to form more of H_3O^+. To measure this kind of acidity, one has to go beyond the normal pH scale.

Clay minerals are hydrous phyllosilicates predominantly composing clays (rocks). These are hydrous silicates of Al, Mg, K, and Fe, and other less abundant elements. Clay minerals are extremely fine crystals or particles, often colloidal in size and usually plate like in shape[25]. The very fine particles yield very large specific surface areas that are physically sorptive and chemically surface active. Many clay mineral crystals carry an excess negative electric charge owing to internal substitution by lower valent cations, and thereby increase internal reactivity in chemical combination and ion exchange. This property makes clay active as catalyst.

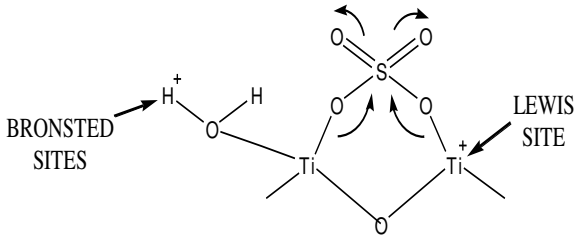
With intercalation of metal complexes into clay, a new property of material will be produced, having higher surface area and particular active sites[26]. The preparation is described as below. Superacid catalysts can be prepared by several approaches: liquid superacids supported on suitable carriers, a combination of metal halides with inorganic salts such as $AlCl_3$-Ti $(SO_4)_3$, $AlCl_3$-$CuCl_2$ etc, per fluorinated resin sulfonic acid such as Nafion-H, and sulfate-promoted metal oxides such as SO_4^{2-}/ZrO_2, SO_4^{2-}/TiO_2, SO_4^{2-}/Fe_2O_3[12-20,25-31].

Among these, sulfate-promoted metal oxides have been found to exhibit excellent catalytic properties for a number of acid-catalyzed hydrocarbon reactions. These catalysts, especially those of the sulfated zirconia type, are able to catalyze the isomerization of short linear alkanes at relatively low temperature (below 150 ^oC)[27]. Even though it is accepted that the presence of sulfate species with covalent S=O bonds on the oxide surface is necessary to obtain superacidity[6], the exact nature of the catalytically active sites remains an open question in the literature.

Thus, it is suggested that the superacid centers are Lewis sites associated to the metal cation[7], whose acid strength is strongly enhanced by an electron induction effect of S=O in the sulfur complex, as is shown in figure 3, scheme 1. Others have suggested that the Lewis and Bronsted sites generated from adsorbed water molecules (Figure 3, scheme 2) are responsible for the catalytic activity. These Bronsted sites are easily interconverted to Lewis sites by evacuation at temperatures above 150 ^{o}C.



Scheme 1



Scheme 2

Figure 3. Formation of superacidcatalyst[26]

Synthesis of eugenol dimer from clove oil, which has known to have less cytotoxity and higher anti-inflammatory activity than that parent eugenols[19,20]. The dimerization is an oxidative phenolic reaction, which yields many undesired side products[19]. Therefore, a highly selective nanocatalyst is needed for the efficiency of synthesis. In designing and synthesizing new solid inorganic catalysts the aims are to maximize surface area, activity, selectivity, longevity, and durability.

Application of these catalysts in the nanoscale for future processes and synthesis will develop the nanotechnology field and industrial sector, particularly the production of pharmaceuticals. In this project, Eugenol dimer from clove oil will be provided throughout organic syntheses, and subjected into body samples, in order to find out whether they have greater anti-oxidant and anti-inflammantory activity that parent eugenol. Fujisawa et al. (2004) simulated the dimerization by computer-aid synthesis and concluded that they have higher anti-inflammatory activity[16].

Reaction Mechanism of dimerization of Eugenol

Reaction mechanisms that occur in the process of dimerization of eugenol is called oxidative coupling reaction in which the formation of free radicals in the compound eugenol eugenol compounds for coupling two radicals into a single unit called eugenol dimer. The mechanism of free radical reactions eugenol dimer formation through reaction stages are initiation (beginning), propagation (propagation), and termination (termination)[29]. Marques et al. (1997) revealed that functional groups OH is attached to the bezene ring (phenolic group) has an important role in the oxidative coupling reaction. Molecules that do not have functional groups OH dimerization reaction will not occur or takes a very long time in order to form a dimer[30]. The mechanism of the reaction is as follows:

a). Initiation, i.e. separation into two homolytic H_2O_2 free radical HO*

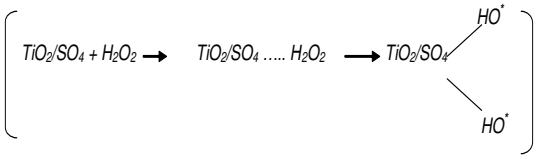


Figure 4. Reaction Mechanism of Free Radical Initiation Phase[30]

The catalyst forms a complex with hydrogen peroxide which is then split into

two hydroxyl radicals. Two hydroxyl radical is attached to the metal cluster peroxide so that it will produce a reaction intermediate. According to the 2004 study, hydroxyl radicals will oxidize phenolic compounds into phenoxy radicals which are quite stable because it can resonate compared with methyl or alkyl radicals[31]**.** The reaction is favored by radical dimerization reaction to form. Dimerization reaction can produce a number of dimeric dehydrogenase of phenoxy radicals, due to the unpaired electrons, which are on oxygen. The ability of the catalyst breaks down the hydrogen peroxide compounds are attached to the metal catalyst. These metals act as an electron storage media from the H_2O_2 thus losing stability and turned into two compounds. OH radical that there is one unpaired electron[32]**.** The presence of substituent OCH_3 (methoxy) and vinyl (tails eugenol) leads to steric factors (obstacle space) so the possibility of coupling the position is smaller than the ortho position of the OH group has a lower steric factors. Low level of steric factors would be very easy to react with other compounds so that the condition of the ortho position of the phenoxy radical will meet with the other ortho position and formed two monomer compounds[30].

b). Propagation**,** namely the propagation of free radicals in the main compound of the free radicals formed during initiation. Phenoxy radical resonance mechanism in all parts of the compound eugenol as shown in Figure 5.

c). Termination**,** the termination of free radical reactions in the presence of inhibitors that react with reactive free radicals in the same position so as to form reactive free radicals and relatively stable. The mechanism of free radical reactions in the termination phase can be seen in Figure 6.

According to Dias in 1998[33], although from the phenolic reaction it has been established that the coupling is always in an ortho or para position to the phenolic hydroxyl group, observation was also conducted to see the possibity of other combinations, namely O-p and O-O. However, the combination is generally not possible because of the instability of peroxide produced, their selectivity of the catalyst and the presence of steric factors.

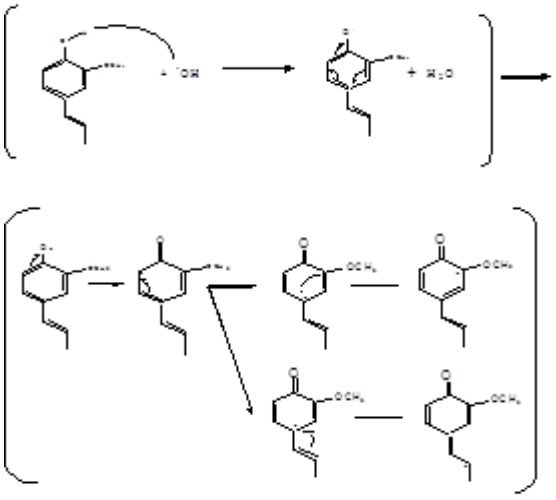


Figure 5. Resonance Mechanism in Phase Free Radical Propagation[29]

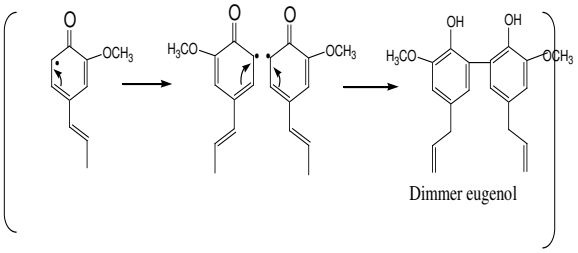


Figure 6. Reaction Mechanism of Free Radical Termination Phase [29]

Vanillin

Some ways of making vanillin has been known. One of the procedures used to produce vanillin in a large scale is a method of CIBA. In this method eugenol reacted with KOH, nitrobenzene in the autoclave. $C_4H_{10}O_3$ solvent used to carry out the isomerization of eugenol into isoeugenol with KOH, and dimethylsufoxide (DMSO)

was used as a medium for the oxidation of isoeugenol to vanillin with KOH and nitrobenzene. Purification of vanillin initially performed in a vacuum distillation and then recrystallized from hot water[28]. The reaction is shown in Figure 7.

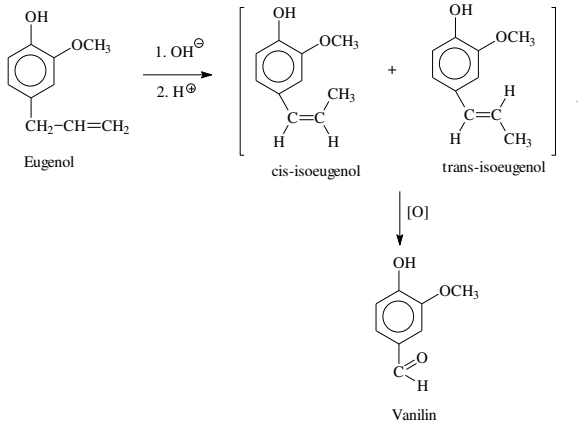


Figure 7. Reaction formation of vanillin[28]

Eugenyl ether

Most common methods for converted hydroxy compounds into ether derivative namely the reactions of metal, usually sodium salts, the alkyl halide or halides arakyl. This reaction is known as the Williamson ether synthesis. In the following discussion first presented eugenol converted into sodium eugenoxide by reaction with a solution of alcoxide in alcohol. Following ether compound formed by the reaction using alkyl halides or arakyl, sometimes the reaction takes place at room temperature.

$$RO^{-}Na^{+} + R'X \rightarrow RO - R' + NaX \quad (2)$$

R' is a primary alkyl group or a benzyl group and X is A, Br or Cl

Formation eugenyl methyl ether (4-Allyl-veratrol, methyl eugenol)

Eugenyl methyl ether is very widely used as a perfume composition with oriental characters. It smells softer than eugenol, which is why the agriculture methyl ether can be used to attract male fruit flies *Docus dorsalis*. By using the method was prepared by Sastrohamidjojo, (1981)[34] known that the first peak is eugenol (81.5 %). The second peak is 1-allyl-3-ethyl-4-hydroxy-5-methoxy benzene (18 %) which is a byproduct of the methylation reaction. Results eugenyl approximately 90.6 % methyl ether theoretically if eugenol and adverse outcomes can be obtained taken into account.

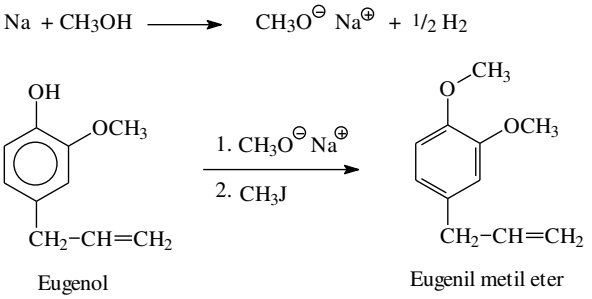
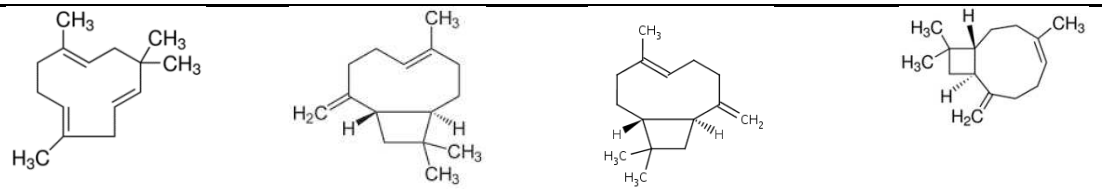


Figure 8. Reaction Formation Eugenyl Methyl Ether[12,35]

Isolation caryophyllene

The top layer of the reaction product of clove oil and NaOH were already separated with the bottom layer containing caryophyllene extracted with petroleum ether. After the petroleum ether removed, the residue was distilled under containing caryophyllene by reduce pressure. Fraction with boiling point 85-102 ^{o}C/6 mmHg accommodated (crude caryophyllene). Originally distinguished name caryophyllene above three names, namely α-, β-and γ- caryophyllene[36]. A-name is replaced with the name caryophyllene humulen (I), β- caryophyllene called caryophyllene (II), γ-caryophyllene called isocaryophyllene (III) as shown in Figure 9.

Caryophyllene was analyzed by using a combination of gas chromatography and mass spectrometry showed eight peaks. Six peaks were identified[37]. The main component is a β- caryophyllene.



Humulen	Caryophyllene	β-caryophyllene (II)	Iso Caryophyllene (III)

Figure 9. Structure of Caryophyllene[36]

Formation of Some Caryophyllene Derivatives

Conversion caryophyllene to caryophyllene oxide. In general epoxidation performed using peroxybenzoat acid, φ-CO_2OH in $CHCl_3$. The caryophyllene can be converted into oxide by adding m-chlorobenzoic acid in CH_2Cl_2. Furthermore, if caryophyllene reacted with Al_2O_3 in the petroleum ether media to be obtained three alcohol mixture[18].

Conversion caryophyllene oxide can be kobusan using ozone[38]. Kobusan can then be converted into an aldehyde mixture which has a woody musky odor by a series of reactions. Hydrolysis is catalyzed by H_2SO_4 to kobusan will produce a glycol. Glycol is obtained when oxidized with chromic acid will be produced oxoalcohol which later turned into diketone. The process is carried out by Barton et al (1953)[39] and Fitjer et al (1995)[40].

Research conducted by Yang and Dienar (1994)[41] showed when caryophyllene oxide was refluxed for 3 hours in a solution of NaOAc / HOAc (pH 4) obtained six compounds. Analysis by GC-MS showed that the first four compounds have a molecular ion, M^+ = m / z = 220, while the last two compounds have a molecular ion M^+ = m / z = 238. Asep, et. al, (2001) have done caryophyllene reaction with O_2 by varying the temperature and reaction time[42]. Conversion caryophyllene to caryophyllene oxide obtained optimum results 84.1 % when the reaction is performed at a temperature of 80 °C for 3.5 hours. Asep et al (2001)[42] also perform synthesis using basic materials of caryophyllene and caryophyllene oxide to caryophyllene alcohol. In this study, using formic acid, acetic acid, and butyric acid.

Derivation of Clove Oil by Biocatalysis

In general biocatalysis or biotransformation can be defined as the use of biological systems (whole cells, cellular extracts or isolated enzymes) to catalyze the conversion of one compound to another[43-46]. Similar to chemical catalysts, biocatalysts increase the speed in which a reaction takes place but do not affect the thermodynamics of the reaction[45]. Fermentation to produce alcohol and cheese production from milk protein was the examples of biocatalysis process that have been used since ancient time[45]. There is an increasing trend of investigation toward the utilization of biocatalysis potential for production value added products from conventional as well as nonconventional substrates[46]. In Europa Bio 2003, biocatalysis represent the main pillar of applied biotechnology, which has been coined as "White Biotechnology" and which stands for the application of Nature's toolset to sustainable industrial production[47]. Advantage of biocatalysis compare to chemical catalysis includes high chemo-, regio-, and stereo-selectivities; require mild reaction conditions; normally performed in an aqueous environment but can, in many cases, also be conducted in solvent mixtures, liquid– liquid two-phase

systems, and even in pure organic solvents; there is no, or only limited use of protecting groups; when using whole cells as biocatalyst, more than one reaction can be accomplished as cell cultures can express a series of enzyme activities; the process of biocatalysis may be simple where the process is mediated by one or more enzymes with many steps; Single step biotransformation is comparatively efficient, as the yield decreased with increase in steps; natural or synthetic substrate can be used in biocatalysis; besides biocatalysis consider as more environmentally friendly[48-54].

Biocatalysis using plant and microorganism enzymes system

Dimerization biocatalysis process of clove oil derivatives based on reaction using horseradish peroxidase (HRP) and H_20_2 (Figure 10) follow by reaction with laccase to oxidise residual H_20_2 can be conducted[49-50]. The biocatalysis relied on HRP to oxidatively dimerise eugenol within the essential oil, this condition change the liquid soluble component into an unsoluble solid material, the product could be separated by filtration[49-50]. Using this process, biocatalysis to form eugenol dimer[51-52] and isoeugenol dimer[51] have been reported. Other plant peroxidase enzymes preparation, such as from Indonesian vegetable "sawi hijau"(*Brassica juncea)*[53] and from callus[54] of alfalfa, arnica, gotu kola and bean. *B. juncea* peroxidase could also be used for dimeriztion of guaicol[55], whereas the callus of alfalfa, arnica, gotu kola and bean also reported for dimerization of vanillin[54].

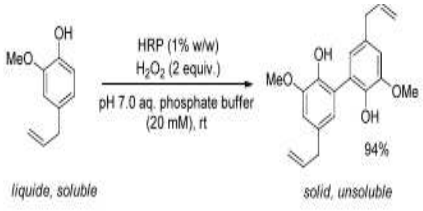


Figure 10. Dimerization of eugenol using HRP catalysis in the presence of H_2O_2[40]

Wu et al. reported studies on biocatalysis of clove oil into vanillin using soybean lipoxygenase (SBLOX) (Figure 11) as biocatalyst in a silicon rubber membrane bioreactor (SRMBR) and shaking flasks. The results showed that the vanillin conversion in SRMBR (121.53 mg/L) was much better than the conversion in shake flask (8.14 mg/L) after 36 h, whereas the conversion rate of clove oil was 0.033 % in the shaking flask and 1.01 % in the SRMBR[56].

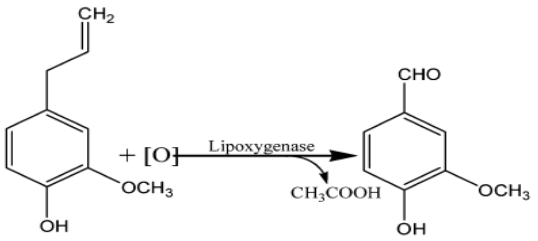


Figure 11. Bioconversion of eugenol into vanillin catalyszed by SBLOX[55]

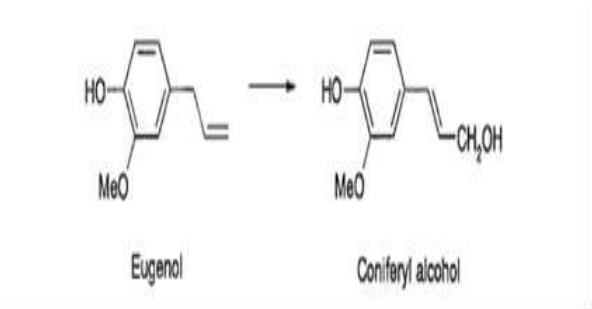


Figure 12. Biocatalysis of eugenol into coniferyl alcohol by eugenol dehydrogenase[56]

Microbial source also shows activities of several enzymes that could act as biocatalyst for biotransformation of clove oil derivatives. Eugenol dehydrogenase, the enzyme that catalyzes the conversion of eugenol to coniferyl alcohol (Figure 12) has been isolated and purified from *Pseudomonas fluorescens* E118[56]. Activities of eugenol hydroxylase, feruloyl-CoA synthetase, vanillate-O-demethylase, and protocatechuate 3,4-dioxygenase have

been detected in cell free extract of thermophilic *Geobacillus* sp. AY 946034 strain grown on eugenol[57].

Biocatalysis process using plant cell cultures system

Plant cell cultures from nine plant species have been used to study the biocaltalysis system of eugenol and isoeugenol to form the dimers via the oxidative coupling reaction to develop an alternative to chemical methods for the synthesis of the bioactive compounds[58]. The species used are: alfalfa (*Medicago sativa* L.), bean (*Phaseolus vulgaris*), coriander (*Coriandrum sativum*), *Psacalium peltatum*, melon (*Cucumis melo*), carrot (*Dacus carota*), *Prunus serotina*, *Mammillaria huitzilopochtli* and *Bouvardia ternifolia*. The results showed that the all cell suspension cultures could transformed eugenol to produced dehydrodiugenol as the sole major product which coriander (*C. sativum*) produced the highest yield of dehydrodieugenol (35 %)[58]. Biocatalysis of eugenol to form biseugenol was also reported using *Kalopanax pictus* cell cultures which could produced 16.3 mg/L of biseugenol[54][59]. Plant cells cultuted of *Eucalyptus perriniana* showed ability as biocatalyst for glycosylation reaction of isoeugenol and eugenol[60]. There were 5 biocatalysis products of isoeugenyl (Figure 13) and 3 biocatalysis product of eugenol (Figure 14)[60]. Later Shimoda et al. (2006)[61]. reported that this plant cell cultured could transform eugenol to give yield of 7 % eugenyl beta-glucoside and 58 % eugenyl beta-gentiobioside[61]. Glycosilation ability in biocatalysis of eugenol also reported in crown galls of *Panax quinquefolium* that could transform eugenol into 3 biocatalysis product namely 2-methoxy-4-(2-propenyl)phenyl-O-b-D-glucopyranoside (67.11 %), 2-methoxy-4-(2-propenyl) phenyl-O-b-D-glucopyranosyl-b-D-xylopyranoside (2.85 %) and methyl eugenol (14.30 %) and cell suspension cultures of *Nicotiana tabacum* that only could transform eugenol to 2-methoxy-4-(2-propenyl)phenyl-O-b-D-glucopyranoside[62].

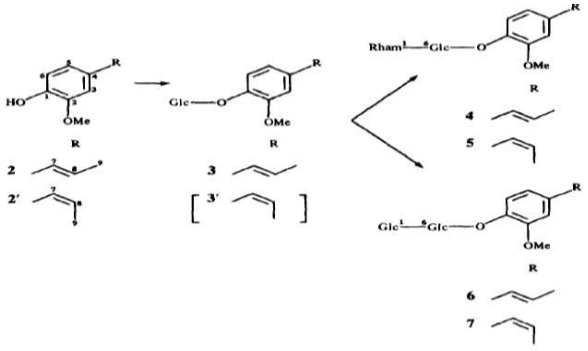


Figure I3. Biocatalysis of isoeugenol (cis and trans ratio 20:1) by *E. perrinzana* cell suspension cultures[60]. Trans isoeugenyl-beta-glucoside (3), trans-amd cis- isoeugenyl beta-rutinoside (4 and 5), trans-and cis isoeugenyl beta-gentiobioside (6 and 7)

Biocatalysis Using Microbial Cultures System

Currently there is an increase in demand for natural products, which lead to increase in studies of microbial as biocatalysis to convert simple metabolite into valuable metabolites that used in food and pharmaceutical industries. It has been reported that important value-added products such as coniferyl alcohol, coniferyl aldehydes, ferulic acid and vanillin were produced by microorganisms as biocatalysis product of eugenol[45]. Figure 15 show the pathway of microbial biocatalysis of eugenol to vanillin. Screening on enrichment culture applying eugenol as a sole carbon source resulted 8 eugenol-degrading microorganisms whereas *Bacillus* sp. strain BR showed the highest vanillin production. This microbe could act as biocatalyst to convert 0.0128 mM eugenol to 0.32mg/l vanillin after 48 hour incubation[63].

P. fluorescens E 118 cultures could act as biocatalyst of eugenol and clove oil to produce ferulic acid [64]. Using 26.6 g of

clove oil resulted 11.6 g of ferulic acid in 2 L of culture broth[64]. . *Geobacillus* sp. AY 946034, a thermophilic bacterial strains was reported could grown in the media with eugenol lower than 0.02% and showed that the major metabolites of biotransformation products are protocatechuic acid, vanillic acid, coniferyl alcohol and ferulic acid[57]. Studies of biocatalysis of eugenol to vanillin have been conducted using genetic engineered microorganism. Recombinant strain *E. coli* XL1-Blue (pSK*vao*mP*calA*m*calB*) converted eugenol to ferulic acid whereas *E. coli* (pSK*ech*E/H*fcs*) was used to convert ferulic acid to vanillin[65]. . There are many other report of the use of microbial systems as biocatalysis of eugenol as shown in Table 2[45].

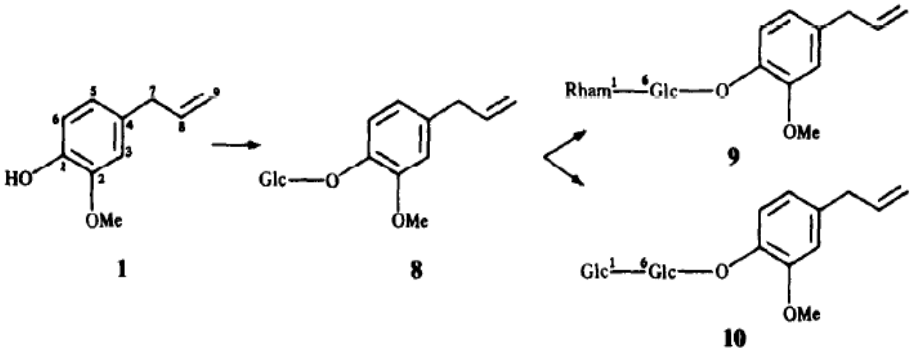


Figure 14. Biocatalysis of eugenol by *E. perrinzana* cell suspension cultures[60]. Eugenyl beta–glucoside (8). Eugenyl beta rutinoside (9) and eugenyl beta-gentiobioside (10)

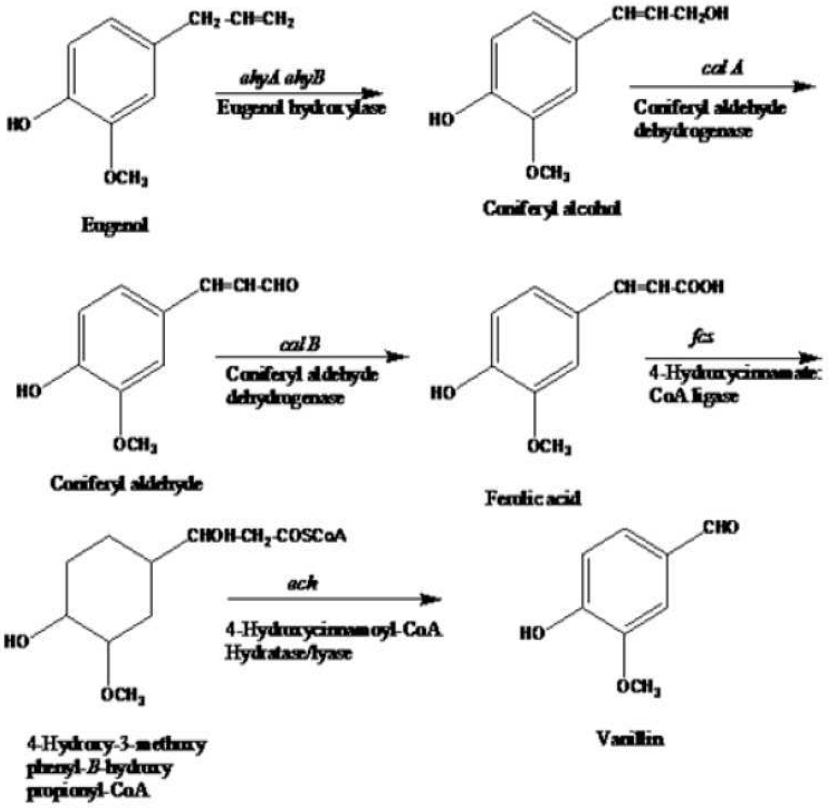


Figure 15. Microbial biocatalysis of eugenol to vanilin[63]

Table 2. Microorganisms involved in biocatalysis of eugenol[45]

Microorganisms	Products
***Corynebacterium* sp.**	**Ferulic acid, vanillin, vanillic acid, proocatechuic acid and keto adipic acid**
***Pseudomonas* sp.**	**Eugenol oxide, eugenol-diol, coniferyl alcohol, coniferyl aldehyde and ferulic acid**
***Enterobacter* sp.**	**Vanillin**
***Pseudomonas* sp.**	**Coniferyl alcolohol, coniferyl aldehydes, ferulic acid and vanillic acid**
***Pseudomonas* sp. HR199**	**Ferulic acid, coniferyl alcohol, coniferyl aldehyde, vannilin and vanilic acid**
***Ralstonia eutropha* H16 (GM)**	**Coniferyl alcolohol, coniferyl aldehydes and ferulic acid**
***Escherichia coli* (GM)**	**Coniferyl alcolohol, coniferyl aldehydes, ferulic acid, and vanillin**
***Pseudomonas fluorescens* E118**	**Coniferyl alcolohol**
***Amycoltopsis* sp. HR167 (GM)**	**Coniferyl alcolohol, coniferyl aldehyde, ferulic acid, guaiacol and vanillic acid**
***Pseudomonas nitroreducens* Jin1**	**Coniferyl alcolohol, ferulic acid, vanillin and vanillic acid**
***Bacillus cereus* PN24**	**4-vinyl guaiacol, vanillin, vanillic acid, protocatechuic aid and keto-adipic acid**
***Streptomyces* sp.**	**Coniferyl alcolohol, ferulic acid and vanillin**
***Pseudomonas resinobrans* SPR1**	**Coniferyl alcolohol, coniferyl aldehyde, ferulic acid, vanillin and vanillic acid**
***Pseudomonas* sp. OPS1**	**Coniferyl alcolohol, coniferyl aldehyde, ferulic acid, vanillic acid and protocatechuic acid**

Note: GM=genitically modified

CONCLUSION

This review summarize information on various clove oil derivatives as well as various processes of clove oil derivatives using chemical catalysis and biocatalysis that will be useful to understand the current status and future prospect of many clove oil derivatives that was used as *chemical industry aromatic clove oil derivatives* in food, fragrance and pharmaceutical industries.

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