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Research Paper

Chemical Transformation of Eugenol Isolated from leaves of *Syzygium aromaticum* to its new isothiocyanate derivatives

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ABSTRACT

The main aim of this research was to develop new or novel compounds with potential biological activity from readily accessed natural products, in particular eugenol. Eugenol (**1**) has been previously isolated from leaves of *Syzygium aromaticum* and transformed to its nitro eugenol in good yield by adding potassium hydrogen sulfate and ammonium nitrate. The nitro group of eugenol reduced smoothly to amino-eugenol by treated with zinc and formic acid. Reaction of amino eugenol (**3**) with carbon disulfide which mainly analysed by GC-MS produced eugenol (**1**) (22.46%), $M^{+\cdot}$ 164; isothiocyanate derivative (**4**) (4.36%), $M^{+\cdot}$ 221; amino eugenol (**3**) (32.16%), $M^{+\cdot}$ 179; thiolisothiocyanate (**5**) (4.28%), $M^{+\cdot}$ 256; unidentified compound (4.04%); major isothiocyanate derivative (**6**) (32.70%), $M^{+\cdot}$ 221.

Keywords: Chemical transformation; Eugenol; New isothiocyanate.

INTRODUCTION

The discovery, development, and application of natural drugs has been done for thousands of years. As a type of plant extracts, eugenol (**1**) as a very wide biological activities and application history. The aim of this research was to develop new or novel compounds with potential biological activity such as isothiocyanate derivatives from readily accessed natural products, in particular eugenol. Isothiocyanate is sulphur-containing phytochemicals and has the chemical group -N=C=S, formed by substituting the oxygen in the isocyanate group with a sulfur. Isothiocyanates have been shown to be especially effective in fighting cancers (Cinciripini et al., 1997; Hecht, 2000). Isothiocyanates can be found in cruciferous or "cabbage family" vegetables such as broccoli, cauliflower, kale, turnips, collards, Brussels sprouts, cabbage, kohlrabi, rutabaga, Chinese cabbage, bok choy, horseradish, radish, and watercress (Drewnowski and Carneros, 2000). The synthesis of isothiocyanates proceeds through the reaction between a primary amine (e.g. aniline) and carbon disulfide in aqueous ammonia. This results in precipitation of the ammonium dithiocarbamate salt, which is then treated with lead nitrate to yield the corresponding isothiocyanate (Dains et al., 1926). Isothiocyanate derivatives could be also

synthesized from readily accessed natural products (e.g. eugenol) which is easily isolated from *Syzygium aromaticum*.

Cloves (*Syzygium aromaticum*) are harvested primarily in Indonesia, Madagascar, and Zanzibar, India, Pakistan, and Sri Lanka. According to FAO, Indonesia produced almost 80% of the world's clove output in 2005. Cloves contain eugenol (4-Allyl-2-methoxyphenol), a main constituent of the essential oil and have been used for antimicrobial (Eyambe et al., 2011) and anesthetic (Jadhav et al., 2004). Eugenol is considered as a phenolic compound similar to benzene with three substituents (hydroxy, methoxy, and allyl) which undergo electrophilic aromatic substitution reactions through nitration (Sudarma et al., 2014). Nitro-eugenol is of considerable importance in the production of other fine chemicals such as amino-eugenol for further chemical synthesis. Nitro compounds can be reduced easily to the corresponding amino derivatives. Amino eugenol has an amino group (-NH_2) which is also easily reacted with acyl and carbon disulfide. Eugenol has been previously investigated and reported for its further chemical transformation (Sudarma et al., 2009a; 2009b; 2013; Sudarma, 2010; Carrasco et al., 2008), but its transformation to new isothiocyanate derivatives has not been reported yet.

MATERIALS AND METHODS

Materials: The material used included: dry fall leaves of clove (*Syzygium aromaticum*) were collected in july 2012, in Gangga village (northern of Lombok), dichloromethane, hexane, methanol, tetrahydrofuran, sodium hydroxide pellet, acetonitrile, ammonium nitrite, potassium hydrogen sulfate, zinc powder, formic acid, silica gel, sodium carbonate anhydrous, acetyl chloride, carbon disulfide, analytical thin layer chromatography.

Instrumentation: GC-MS were recorded on GC-MS QP-5050A, BC-17A and MS 5050A Merk Shimadzu. The original ^{1}H nmr, ^{13}C nmr, and DEPT spectra are directly reproduced throughout. They were generally recorded in $CDCl_3$ on a Bruker spectrometer at 400MHz.

Procedure: Dried leaves of clove was chopped and grounded to fine particles (125g) and percolated with dichloromethane (250ml) and kept for 24 hours. Then the liquid extract was filtered and evaporated to afford yellowish oil (12.5g). This oil was analyzed by GC-MS to confirm the presence of eugenol. Eugenol was obtained from the leaves clove oil, according to standard procedure and identified by GC-MS and NMR analyses. $M^{+\cdot}$ 164, cal for $C_{10}H_{12}O_2$ Major fragments: 49 (M+. –CH_3), 131, 121, 103, 91, 77 (C_6H_6, base peak). ^{1}H NMR (400.1 MHz, $CDCl_3$): δ 3.35 (2H, d, J 6.6 Hz, H1'); 3.93 (3H, s, OCH_3); 5.13 (2H, m, H3'); 5.50 (1H, s, OH); 5.91 (1H, m, H2'); 6.5 - 6.96 (3H, aromatic protons).

***Nitration of eugenol using NH_4NO_3/$KHSO_4$*:** A round bottomed flask (50ml) with magnetic stirrer was charged 1.00g eugenol (6.10mmol) and acetonitrile (20ml) then stirred for 5 min. Potassium hydrogen sulfate (0.64g) and ammonium nitrate (1.4g) were added and stirred at room temperature for 30 min then refluxed for 5 hour. Worked up was based on Baghernejad et al., 2009, to afford yellowish to redish oil (1.1g, 86.28%, pure by tlc analysis). Compound **(2)** (oil): $M^{+\cdot}$ 209, cal for $C_{10}H_{11}NO_4$ Major fragments : 195 ($M^{+\cdot}$ –CH_2), 178, 163, 147, 131, 119, 103, 91 (base peak). IR (film) n_{max}/cm^{-1}: 3232 (O-H), 3084 (C=CH-Ar), 3014 (CH=CH_2), 2936, 2829, 1634 (C=C), 1547 (NO_2), 1399, 1327, 1260 (C-O), 1127 (C-O), 1066, 999, 912, 764. ^{1}H NMR (400.1 MHz, $CDCl_3$): δ 3.35 (2H, d, *J* 6.6 Hz, H1'); 3.93 (3H, s, OCH_3); 5.13 (2H, m, H3'); 5.91 (1H, m, H2'); 6.96 (1H, s, H3); 7.50 (1H, d, *J* 0.9 Hz, H5); 10.67

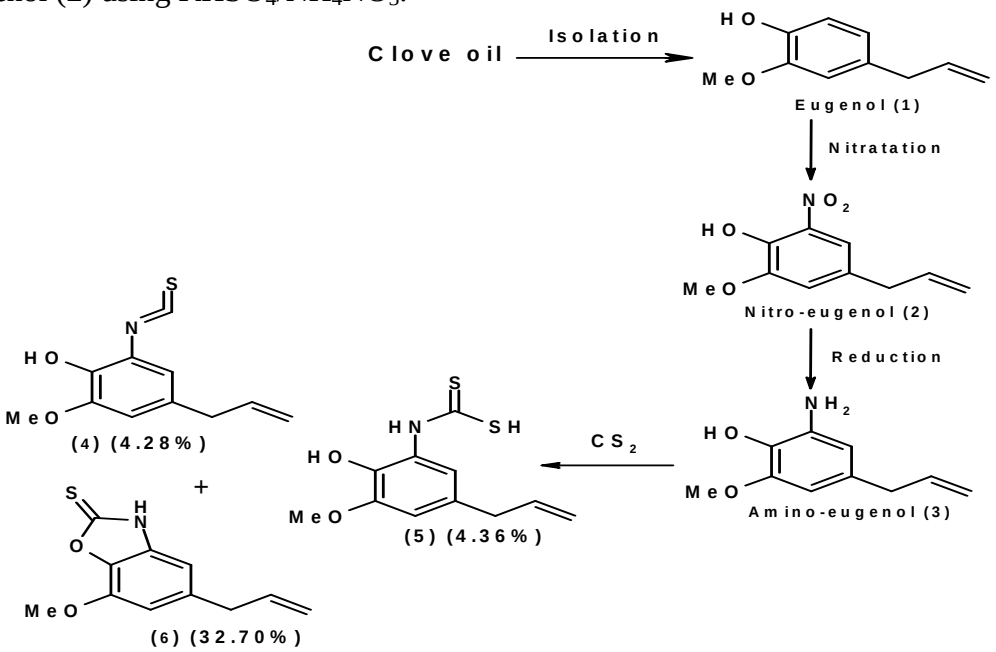
(1H, s, OH). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 39.4 (C1'); 56.7 (OCH3); 115.1 (C5); 117.1 (C3'); 118.6 (C3); 131.2 (C4); 133.6 (C6); 135.9 (C2'); 144.9 (C1); 149.8 (C2).

***Preparation of amino-eugenol (3)*:** Nitro-eugenol (620mg, 2.96mmol) was dissolved in methanol (10ml) and the solution stirred with zinc powder (1g) and formic acid (2.5ml) for 10 minute. Worked to afford the desired amino eugenol compound (85% yield) (Gowda et al., 2001). GC-MS: M+. 179, cal for $C_{10}H_{13}NO_2$ Major fragments: 164, 152, 136, 118, 106, 91 (base peak). ^{1}H NMR (400.1 MHz, $CDCl_3$): δ 3.21 (2H, d, CH_2), 3.48 (1H, s, NH); 3.68 (1H, s, NH), 3.85 (3H, s, OCH_3); 5.05 (2H,m,CH_2), 5.25 (1H,s,OH); 5.91 (1H, m, H2'); 6.18 (1H,s, ArH), 6.25 (1H,s,ArH). FT-IR cm^{-1}: 3389 (O-H), 3306 (NH_2), 2943 (C=CH-Ar), 2848 (CH=CH_2), 1608 (C=C), 1131 (C-O)

***Carbon disulfide reaction of amino eugenol*:** Amino eugenol (250mg, 1.40 mmol) was dissolved in a minimum of tetrahydrofuran and stirred for 10 min, then carbon disulfide (0.2 ml) was added. The solution was stirred overnight. The precipitate was filtered and washed by methanol to afford amorphous solid (245mg). GC-MS analysis gave eugenol (**1**) (22.46%), isocyanate derivative (**4**) (4.36%) $M^{+\cdot}$ 221; amino eugenol (**3**) (32.16%); thiolisothiocyanate (**5**) (4.28%) $M^{+\cdot}$ 256; unidentified compound (4.04); major isothiocyanate derivative (**6**) (32,70%). GCMS: $M^{+\cdot}$ 221, cal for $C_{11}H_{11}NO_2S$ Major fragments: 206 ($M^{+\cdot}CH_3$), 179, 164, 147, 131, 118, 91, and 77.

RESULTS AND DISCUSSION

Herein, we report our results on Chemical Transformation of Eugenol Isolated from leaves of *Syzygium aromaticum* to its new isothiocyanate derivatives (Scheme-1). In the synthetic approach based on amino-eugenol (**3**) as a main material for the synthesis of isothiocyanate derivatives, required nitro-eugenol (**2**) as a precursor. This was prepared by isolation of eugenol (**1**) from clove oil then converted to nitro-eugenol (**2**) using $KHSO_4/NH_4NO_3$.



Scheme-1: Synthesis of new isothiocyanate derivatives from eugenol (1).

The structure of the isolated eugenol (**1**) and nitro-eugenol (**2**) were confirmed by their mass spectrum and ^{1}H NMR. The GC-MS analysis of (1) showed the molecular ion at m/z 164, consistent with the molecular formula $C_{10}H_{12}O_2$. The ^{1}H NMR.

spectrum of (**1**) confirmed 12 protons, with the methoxy singlet at 3.93 ppm; three aromatic proton of benzene ring at 6.5 - 6.96 ppm; one phenolic proton at 5.50 ppm; and the remaining five allyl proton at 3.35, 5.13, and 5.91 ppm. The GC-MS analysis of (2) showed molecular ion at m/z 209, calculated for $C_{10}H_{11}NO_4$ The FT-IR of (2) gave a characteristic –OH phenol dan nitro groups stretching bands at frequency 3232 (O-H), and 1547 (NO_2). The ^{1}H NMR. spectrum of (2) based on Carrasco et al., 2008, gave signal –OH shifted downfield at 10.67 ppm due to the formation of hydrogen bonding between hydrogen from –OH with oxygen from NO_2.

Further stage in the synthesis of the amino-eugenol (**3**) was to reduce the nitro-eugenol (**2**) to the corresponding amine. Typical reducing agents such as tin/HCl, Zn/$CaCl_2$, Zn/formic acid have been used for the reduction of nitro aromatic (Godwa, et al., 2001). It has been reported that nitro aromatic compound was reduced smootly with zinc powder and formic acid in methanol to afford amino-group. The structure of amino-eugenol (**3**) was confirmed spectroscopically. The GC-MS analysis observed molecular ion at m/z 179 consistent with the molecular formula $C_{10}H_{13}NO_2$. Comparison of the ^{1}H NMR spectra of (**2**) and (**3**) revealed that the chemical shifts of the protons were very similar except the amino group of (**3**) showed a two protons singlet at 3,48 and 3,68 ppm. The remaining protons were assigned to the phenolic as a signal at 5.25 ppm, a methoxy at 3.85 ppm, the protons of methylene which attached on benzene ring at 3.21 ppm, and a methylene terminal at 5.05 ppm. An olefinic proton gave signal at 5.91 ppm and two aromatic protons gave signal at 6.18 and 6.25 ppm. In the FT-IR spectrum of (**3**) a characteristically sharp bands for the aromatic primary amine group was present at slightly higher frequencies (shorter wavelength, 3306 cm^{-1} compare to aliphatic primary amine (Silverstein et al., 1981).

Carbon disulfide treated with amino-eugenol (**3**) for overnight reaction and analysed by GC-MS produced isothiocyanate derivatives (**4**), (**5**), (**6**), unreacted amino-eugenol (**3**), and eugenol (**1**) (**Figure-1**).

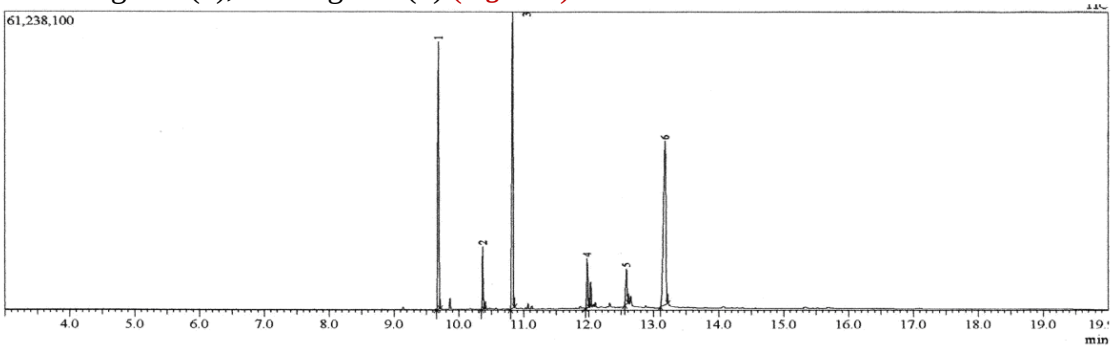
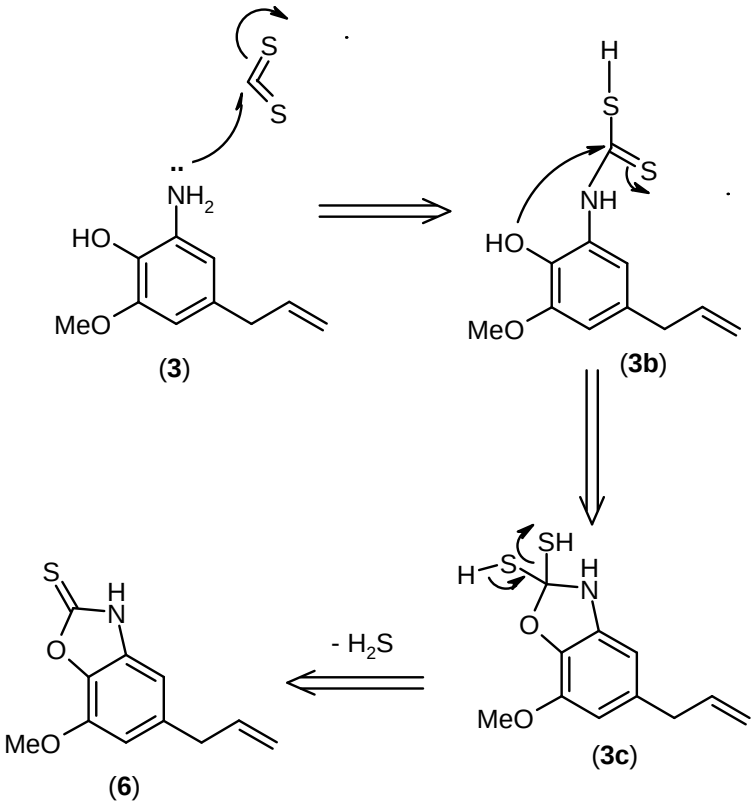


Figure-1: GC-MS analysis the reaction of amino eugenol and CS_2.

The GC-MS analysis showed peak 6 as a major isothiocyanate derivative which has molecular ion at m/z 221 corresponding to the molecular formula $C_{11}H_{11}NO_2S$. Mechanistically, the formation of the compound (**6**) can be explained as follows (**Scheme-2**): nucleophilic attack of the amino group from amino eugenol (**3**) on the carbon of carbon disulfide to produced intermediated (**3b**), followed by hydroksi addition to give (**3c**). Elimination of hydrogen sulfide would then produced (**6**).



Scheme-2: The formation of the compound (6)

The reaction of amino eugenol (**3**) with CS_2 gave side products eugenol (**1**) and unreacted amino eugenol (**3**). These products occur was due to elimination of amino group by acid (H_2S) which was generated during the formation of compound (**6**). This acid (H_2S) could be react with amino group through nucleophilic substitution reaction.

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