

## Chemical Constituents of Essential Oil from the Leaves of Ghanaian *Ocimum gratissimum* L.

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Leaves from Ghanaian *Ocimum gratissimum* yielded essential oil upon distillation. A fraction from the column chromatography supported infrared and proton nuclear magnetic resonance absorption and also GC-MS revealed that thymol is the major component. The melting point of phenylurethane derivative of the fraction from the column chromatography conclusively indicated thymol and not eugenol. Also GC-MS analysis on the steam distillation extract of EtOH : hexane mixture (1 : 1) gave these constituents:  $\beta$ -selinene, terpinene-4-ol,  $\gamma$ -terpinene, terpinolene,  $\alpha$ -terpinene and  $\alpha$ -thujene which are not reported in the earlier studies.

**Key Words:** *Ocimum gratissimum*, Thymol, Eugenol, Carvacrol.

### INTRODUCTION

*Ocimum gratissimum* L. (Labiata) known as *Ocimum viride* Willd<sup>1</sup> is a common plant grown around houses for use in traditional medicine. Phenylurethane and *p*-iodophenylurethane derivatives of the yellow oil were obtained from leaf extract by Route Bertrand<sup>2</sup> from Ivory Coast and Central African Republic at 107 and 175°C corresponding to the literature values of thymol; then he concluded that the yellow oil from the leaves of *O. gratissimum* contained thymol. Glichitch and Naves<sup>3</sup> reported both thymol and carvacrol (an isomer) as components of yellow oil from the leaves of *O. gratissimum* from Central African Republic.

Both El-said *et al.*<sup>4</sup> and Sofowora<sup>5</sup> reported thymol as the main component of Nigerian *O. gratissimum* when the essential oil obtained from the leaves was analyzed by GC-MS. Other components found by Sofowora<sup>5</sup> and Sainbury<sup>6</sup> were  $\alpha$ -pinene, camphene,  $\alpha$ -terpinene,  $\Delta^3$ -carene, myrcene, 1,8-cineole, *p*-cymene, linalool, limonene, camphene,  $\alpha$ -terpineol, humulene, methyliso-eugenol, methyl-eugenol, caryophyllene, ocimene and 1-cadiene.

Tripathi *et al.*<sup>7</sup> reported eugenol as the main component in the essential oil from the leaves of *O. gratissimum* (Clocium) in India. No thymol was found as a component of the oil. The essential oil from the leaves of *O. gratissimum* from Ghana was then needed to be investigated.

### EXPERIMENTAL

Infrared spectra of all samples were recorded using neat samples on Specord M 80 cm Zeiss Jena spectrometer. <sup>1</sup>H NMR spectrum samples were recorded using deuterated chloroform (CDCl<sub>3</sub>) with 1% tetramethylsilane (TMS) as internal reference on Hitachi 60 MHz spectrometer, NMR, R-1200 model.

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**Plant materials:** *O. gratissimum* leaves were collected from T.I. Ahmadiyya Secondary School, Prempeh College and Staff Quarters of Kwame Nkrumah University of Science and Technology (KNUST), Kumasi. The leaves were identified at Herbarium of Biological Sciences Department, KNUST, Kumasi, Ghana.

**Steam distillation:** 6.3 kg of fresh leaves of *O. gratissimum* were chopped into pieces and subjected to steam distillation. Diethyl ether was used to extract the crude oil and dried over anhydrous sodium sulphate. Diethyl ether was removed by simple distillation and the crude oil which was yellow in colour was obtained.

**Thin layer chromatography (TLC):** Microscope slides coated with Macherey Nagel's polygram silica gel (0.25/UV<sub>254</sub>) were used for TLC. The dried *O. gratissimum* oil (0.5 mL) was dissolved in diethyl ether 5 mL and spotted on microscope slides with silica gel. The slides were developed using petroleum ether : toluene mixture (8 : 2 v/v). The separated spots on the developed chromatogram were viewed under iodine vapour and UV light (254 nm). Four separated spots in iodine vapour were identified and the  $R_f$  values calculated.

The dried oil (15.0 g) was put on a glass column packed with silica gel (60.120 mesh) (375 g) and eluted with petroleum ether : toluene mixture (8 : 2 v/v). Drops of the eluate from the column (5 mL) were collected in test tubes. The contents of the test tubes were subjected to TLC and eluted with the same  $R_f$  values as obtained previously were pooled together. Three fractions  $C_1$ ,  $C_2$  and  $C_3$  were obtained in this manner. Each fraction was concentrated on Buchi rotary evaporator and finally dried in an oven at 60°C to a constant weight. A fourth fraction,  $C_4$ , was obtained when the column was eluted with ethanol.

## RESULTS AND DISCUSSION

Fresh leaves of *O. gratissimum* (6.3 kg) yielded 22.9 g (0.36%) of crude yellow oil upon steam distillation; the major component of the oil when eluted with petroleum ether : toluene mixture (8 : 2 v/v) was the second component,  $C_2$ , whose yield was 52%. This component showed as one spot on a TLC with  $R_f$  0.34.

Assignable absorptions in the infrared and  $^1\text{H}$  NMR spectra of  $C_2$  are summarized in Table-1.

TABLE-1

[IR] ( $\nu_{\max}$ $\text{cm}^{-1}$ )	$^1\text{H}$ NMR ( $\text{CDCl}_3$ with 1% TMS $\delta$ ppm)
3600–3200 (B) (OH)	4.88, 1H(b) (Ar—OH)
3020 (w), 1615, 1515 (s) (Ar—H)	6.59–7.01, 3H, M, Ar—H
2930, 1460(s) (— $\text{CH}_2$ , $\text{CH}_3$ )	2.28, 3H, s, Ar— $\text{CH}_3$
1385 and 1375 (s) ( $\text{CH}(\text{CH}_3)_2$ )	1.28, 6H, d, $J = 6.5$ Hz, — $\text{CH}(\text{CH}_3)_2$ 3.11, 1H, m, (— $\text{CH}(\text{CH}_3)_2$ )

The infrared spectrum of  $C_2$  showed a strong C—H absorption at 29.80  $\text{cm}^{-1}$ . A strong doublet absorption representing geminal methyl was observed at 1385–1375  $\text{cm}^{-1}$ . The  $^1\text{H}$  NMR spectrum showed six portion doublet with coupling constant ( $J = 6.5$  Hz) at 1.28 ppm representing two methyl groups. These two methyl groups must therefore be vicinally coupled to a single proton and should therefore be the germinal methyl indicated in the infrared. This assertion is further enhanced by the recognition of methine proton multiplet at 3.11 ppm

which compares with the methine proton septet at 3.15 of the pure thymol spectrum<sup>8,9</sup>. The above information clearly indicates that C<sub>2</sub> contains the isopropyl {—CH(CH<sub>3</sub>)<sub>2</sub>} structural unit. The shift of the methine proton to a lower field is predictably due to the deshielding effect of the aromatic ring current.

The <sup>1</sup>H NMR spectrum also showed a three proton singlet at 2.28 ppm indicating the presence of a methyl group. The low field chemical shift of this methyl singlet when compared to known chemical shift of other methyl singlet also indicates deshielding of the methyl group by an aromatic ring. The weak absorption at 3020 cm<sup>-1</sup> and strong sharp peaks between 1600–1500 cm<sup>-1</sup> in the infrared spectrum inferred the presence of an aromatic entity. This is supported in the <sup>1</sup>H NMR spectrum by a three-proton multiplet from 6.59–7.01 ppm which are exactly the same chemical shift values that can be ascertained by the aromatic protons of pure thymol.

A broad and strong infrared absorption from 3600–3200 cm<sup>-1</sup> suggested the presence of a hydrogen-bonded hydroxyl group. The hydroxyl proton occurred in the <sup>1</sup>H NMR as a broad absorption centred at 4.88 ppm assignable as a phenolic hydroxyl as compared with that in literature<sup>8,9</sup>. A pink phenylurethane derivative formed from the reaction of phenylisocyanate with C<sub>2</sub> melted at 106–107°C supporting C<sub>2</sub> to be thymol. The phenylurethane derivative of an isomeric carvacrol would have melted at 97°C. It can now be established that the major constituent of essential oil from the leaves of *O. gratissimum* in West Africa is thymol. No eugenol has been reported.

The following constituents were found in the essential oil of the leaves of Ghanaian *O. gratissimum* using EtOAc : hexane (1 : 1) which were not found in Nigerian *O. gratissimum* or reported in the earlier studies of *O. gratissimum* using GC-MS. They are β-selinene, terpinene-4-ol, γ-terpinene, α-thujene and α-terpinene

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