



Assessment of the Larvicidal potentials of Thymol derivatives on Anopheles mosquitoes

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ABSTRACT: Thymol (1) a major constituent of the South Eastern Nigeria variety of *ocimum gratissimum* popularly known as nchawu (scent leaf) was converted to its O-methyl (2), O-ethyl (3), acetate (4) and the Benzyloxy (5) derivatives that are characterized by their spectral data such as infra red, proton n.m.r. and mass spectra. Tests on their insecticidal potency show that, like the parent compound thymol (1), they also possess insecticidal properties in decreasing order Benzyloxy > Acetate > O-ethyl > O-methyl. This order is explained partly by the electron withdrawing tendency of the benzyloxy and acetate groups and also that, as the molecular weight of the derivatives increases the solubility decreases. @JASEM

The menace caused by malaria in Nigeria is so devastating that both Government and some organizations have initiated various strategies and programmes for the control of the adult and larvae of the vector (Curtis 1990, Onon 1980 and Matanmi 1991) which causes the disease. In collaboration with the World Health Organization (WHO), Federal Government recently demonstrated a pragmatic approach to the problem of malaria eradication by launching the "roll back malaria." An indigenous plant *Ocimum gratissimum* of the family labiatae (Hutchinson and Dalziel 1987 and Saunders 1958) has been reported to be rich in geraniol (Charles and Simon 1992) and thymol (Bobmanuel and Jack 2004) and also shown to have antimicrobial, insecticidal and fungicidal properties (Awah 1994 and Ofuya 1990). We have harnessed the structure of thymol and prepared its O-methyl (2), O-ethyl (3), acetate (4) and benzyloxy (5) derivatives scheme 1 with a view to determining their suitability or potentials as insecticides/larvicides.

MATERIALS AND METHODS

Infra red spectra were recorded on Pye Unicam SP 1050 spectrometer. ¹H n.m.r., spectra were obtained from varian HA 100 spectrometer using TMS as an internal standard and chemical shifts are given as δ (ppm). Mass spectra were taken on an AETMS 9 double focusing spectrometer at 250°C and 70 eV. All reagents and solvents were purified before use.

Anopheles mosquito larvae were cultured within the main campus of Rivers State College of Education, Rumuolumeni. Five sets of about 2 kg custard buckets were two-third filled with water and left open outside for about two months. By the time of the experimental set up, thousands of larvae were hatched. The buckets were then covered with fine

mesh sized netted material and kept in the laboratory at room temperature of $30 \pm 2^\circ\text{C}$ until use.

An aqueous stock suspension of 25g *Ocimum gratissimum* leaves (also collected on the same campus) in 250ml water was prepared. Based on previous studies (BobManuel and Jack 2004) 20% w/v of the stock suspension was used which was recorded to effect more than 85% mortality of larvae three days after treatment. 2 mls each of Thymol methyl ether (2), Ethyl ether (3), acetate (4) and the benzyloxy derivative (5) in 10ml water were used with 12ml thymol as control.

10 larvae of the same age were counted into each of 15 petri dishes, each treatment being replicated. The treatments were then introduced into each of the Petri dishes with the larvae. Observations and mortality counts were made one hour after treatment on hourly intervals. The experiment terminated within 3 hours due to optimal mortalities observed.

Preparation of Thymol Derivatives

Thymol O-methyl ether (2): 15mls (0.08 moles) of an ethereal solution of diazomethane was added to a solution of 1g (0.07 moles) thymol (1) in 4mls dry tetrahydrofuran (THF) and stirred for 16 hours at room temperature. The solvent was evaporated off and the residue taken up in 60mls chloroform. This was extracted with 2N NaOH (20ml x 2) and acidified to pH 1 with 3N HCl. Re-extraction with chloroform (15ml x 3) and drying over anhydrous Na₂SO₄ gave clear oil.

Yield 0.85g (78%)

¹H n.m.r. see table 2

Mass spectrum M⁺ 164

Thymol O-ethyl ether (3): 500mg (0.003 moles) of (2) in 30ml ethanol was treated with ten drops of

conc. H_2SO_4 and the mixture stirred at room temperature. After 2 hours the mixture was poured into 100ml water and extracted with dichloromethane (15ml x 3), washed with water (15ml x 2) and dried over anhydrous MgSO_4 . Evaporation of the solvent gave oil.

Yield 450mg (83%)
 ^1H n.m.r. see table 2

Mass spectrum M^+ 178

Thymol acetate (4): Into a solution of 1.50g (0.01 moles) thymol (1) in 15ml pyridine was added 8ml acetic anhydride and the mixture stirred at room temperature for 6 hours. It was poured into 100ml ice cold water, allowed to stand for 30mins and extracted with ethyl acetate (15ml x 3). The ethyl acetate extract was washed with 1M HCl (10ml x 2), saturated NaHCO_3 (15ml x 2) and then with water (15ml x 2). Evaporation of the solvent after drying in anhydrous Na_2SO_4 gave an oil.

Yield: 1.60g (84%)
 ^1H n.m.r. see table 2
 Mass spectrum M^+ 192

Benzyloxy derivative (5): A solution of 1.20g (0.008 moles) thymol in 15mls dry acetone was stirred under reflux for 8 hrs. with a mixture of 2.5g (0.02 moles) benzyl chloride and 2.8g (0.02 moles) dry K_2CO_3 . The mixture was cooled and poured into 20mls water and extracted with dichloromethane (20ml x 2), washed with water (15ml x 2) and dried over anhydrous MgSO_4 . Evaporation of the solvent gave yellow oil which was washed with diethyl ether on a short column and obtained as a white gum.

Yield 1.40g (73%)
 ^1H n.m.r. see table 2
 Mass spectrum M^+ 204

Table 1. Effect of Thymol derivatives on Anopheles mosquito larvae

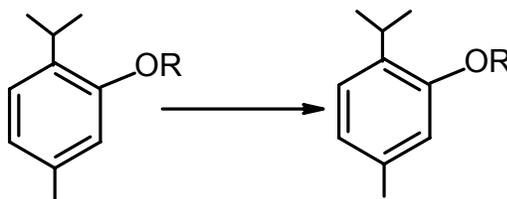
Derivatives	Mean larvae tested	Cumulative Mean Mortality \pm SE	Cumulative percent mortality
Benzyloxy (5)	10	9.8 \pm 3.3	98
Acetate (4)	10	9.1 \pm 2.7	91
Ethyl ether (3)	10	8.8 \pm 2.2	88
Methyl ether (2)	10	8.6 \pm 1.4	86
Thymol (1)	10	8.6 \pm 0.9	86

RESULTS AND DISCUSSIONS

The results obtained are shown in table 1. Cumulative mean mortalities for the Benzyloxy (5) and Acetate (4) were significantly different ($P = 0.05$) compared to the control thymol (1). Cumulative percent mortalities three hours after treatment was also highest for the benzyloxy (98%) followed by the acetate (91%). There were no significant differences between the ethyl (3) and methyl (2) ethers in comparison with thymol. The high cumulative mean mortalities and percent mortalities for the benzyloxy and acetate are indicators that they are potential larvicides. This could be attributed partly to

decreased solubility of these derivatives as the molecular mass increases. It is a known fact that hydrocarbons are generally insoluble in water hence increasing the molecular mass of thymol means increasing the hydrocarbon content with consequent decrease in solubility. These insoluble thymol derivatives by spreading over the water surface obstruct the breathing of the larvae thereby suffocating them or acting as poison. Also the acetoxy and benzyloxy groups are better electron withdrawing groups than the methyl and ethyl groups which are electron releasing groups.

Scheme 1



Where : (1) R = H Thymol; (2). R = CH_3 ; (3). R = CH_2CH_3 ; (4). R = OCOCH_3 ; (5) R = CH_2Ph

Table 2. Proton magnetic resonance spectra*

Protons	Compounds			
	O-methyl Ether (2)	O-ethyl Ether (3)	Acetate (4)	Benzyloxy (5)
H-8 } H-9 }	1.18d (8)	1.14d (8)	1.19d (8)	1.20d (8)
H-10	2.19s	2.18s	2.20s	2.17s
H-7	3.16dq (7.5)	3.18dq (8)	3.17dq (8)	3.16dq (7.5)
OCH ₃	3.70s	-	-	-
OCH ₂ CH ₃	-	3.40q (7)	-	-
OCH ₂ CH ₂	-	1.24t (7)	-	-
OCO CH ₃	-	-	2.23s	-
OCH ₂ Ph	-	-	-	5.05s
ArH	6.51 - 7.07m	6.40 - 6.95m	6.35 - 6.80m	6.49 - 7.10m 7.20 - 7.45m

* Spectra run in CDCl₃

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