

1 **Ovicidal Activity of 2-Hydroxy-4-Methoxybenzaldehyde, Derivatives and Structural**
2 **Analogues on *Anopheles gambiae* eggs**

3

4

5

6

7 **R. E Andati^{1,2}, M. O. Omolo^{1,2*}, I.O. Ndiege³**

8 ***Correspondence Author:** Email: momolo@mmust.ac.ke [MOO]

9

10 ¹Department of Pure & Applied Chemistry, Faculty of Science, Masinde Muliro University of
11 Science and Technology (MMUST), P.O. Box 190, Kakamega 00500, Kenya

12 ²Center For African Medicinal & Nutritional Flora & Fauna (CAMNFF), Masinde Muliro
13 University of Science and Technology (MMUST), P.O. Box 190, Kakamega 00500, Kenya

14 ³Department of Chemistry, Kenyatta University, P.O. Box 43844, Nairobi 00100

15

16

17

18

19

20 **Abstract**

21 **Background**

22 Effective remedies for disrupting *Anopheles gambiae* metamorphosis at the egg stage are crucial
23 in suppression of the malaria vector populations that result in the reduction of disease burden. 2-
24 Hydroxy-4-methoxybenzaldehyde (the major component of *Mondia whytei* roots), its derivatives,
25 structural analogues and their blends were evaluated against the eggs of *An. gambiae* in the search
26 for ovicidal compounds with potential use in mosquito control programs.

27 **Methods**

28 Mature roots were harvested from *Mondia whytei* plants grown in the Center for African Medicinal
29 & Nutritional Flora and Fauna (CAMNFF) herbal medicinal garden and cleaned with distilled
30 water. 2-Hydroxy-4-methoxybenzaldehyde (**1**) was isolated by steam distillation of the chopped
31 roots. The selected derivatives and/or analogues were prepared using established chemical
32 procedures and their structures confirmed by NMR spectroscopy and ESI-MS. Ovicidal activity
33 of the pure compounds, derivatives, structural analogues and/or formulated blends was tested at 1,
34 10, 25 and 50 ppm on *An. gambiae* eggs. .

35 **Results**

36 Eleven mono-substituted (**3-7**), di-substituted (**8-10**), tri-substituted (**1-2**) aromatic compounds
37 were assayed for ovicidal activity against *Anopheles gambiae* eggs singly or as blends.
38 Benzaldehyde (**4**) and 4-methoxybenzaldehyde (**9**) were further converted into 2-hydroxy-1, 2-
39 diphenylethanone (**11**), 1, 5-diphenylpenta-1, 4-diene-3-one (**12**) and 1, 5-bis (4-methoxyphenyl)
40 penta-1, 4-diene-3-one (**13**) and evaluated for ovicidal activity individually or as blends. Of the
41 thirteen compounds evaluated individually, 2-hydroxy-4-methoxybenzaldehyde (**1**) exhibited the

42 highest ovicidal activity at LC₅₀ 0.7075 ppm while anisole had the lowest activity at LC₅₀ 40.342
43 ppm. The derivatives exhibited moderate activity: 2-hydroxy-1, 2-diphenylethanone (LC₅₀ 10.599
44 ppm), 1, 5-diphenylpenta-1, 4-diene-3-one (LC₅₀ 9.019 ppm) and 1, 5-bis (4-methoxyphenyl)
45 penta-1, 4-diene-3-one (LC₅₀ 15.642 ppm). The blends exhibited intriguingly high ovicidal
46 efficacy with the mixture of benzaldehyde and phenol showing the highest (LC₅₀ 0.332 ppm) while
47 phenol and anisole exhibited the lowest activity (LC₅₀ 9.9909 ppm).

48 **Conclusion**

49 From the activity of the blends, it is evident that anisole is antagonistic to the efficacy of phenol
50 and benzaldehyde. It is also apparent that aldehyde and hydroxyl groups, when directly attached
51 to the phenyl ring, provide the critical structural characteristics that contribute to the ovicidal
52 activity of the aromatic compounds.

53 Keywords: 2-Hydroxy-4-methoxybenzaldehyde, derivatives, ovicidal, *Anopheles gambiae*,
54 mosquito, eggs

55

56 **Introduction**

57 Malaria remains the most important parasitic disease in the world [1]. Africa with an estimated
58 215 million annual malaria cases accounts for 94% of the global cases leading to 384,000 deaths
59 [2]. It is estimated that there were 33 million pregnancies in Africa in 2020 with 35% of the
60 expectant mothers being exposed to malaria infection resulting in about 82,000 children with low
61 birth weight [2].

62 Mosquitoes are important public health vectors of malaria, filariasis and arboviral diseases that
63 cause millions of infections and death worldwide [3]. Malaria is transmitted by infected female
64 *An. gambiae* which feed on human blood meal for viability of its eggs [4]. Effective vector control
65 methods at the egg, larval or adult stages are therefore critical in controlling the malaria vector and
66 mitigating its harmful effects on human health [5]. Most malaria control strategies: environmental
67 management (breeding/resting sites), sterile insect technique: biological control agents (predators,
68 parasitoids and entomopathogens); chemical repellents and insecticide/pesticides (natural and
69 synthetic), depend heavily on insect vector population control of the larval or adult stages with
70 little effort on the eggs [6]. .

71 Natural insecticide/pesticides are generally non-pest specific, biodegradable, non-allergic to
72 humans, safe to non-target organisms [7] and have wide spectrum of application [8]. They offer
73 good alternatives to synthetic chemical insecticides which are deleterious to the environment,
74 harmful to non-target organisms and are ineffective due to development of resistance [9]-[13].
75 Many reports of plant extracts, secondary metabolites, essential oils and lectins which exhibit:
76 general insect toxicity; growth and/or reproductive inhibition; insect repellency; and larvicidal
77 activity against mosquito vectors have been documented; and are important and potentially
78 suitable for use in integrated vector management (IVM) [6], [14]. . However, little work has been

79 documented on ovicidal activity and oviposition deterrence of anti-mosquito plants and/or derived
80 compounds.

81 In that regard, the ovicidal activity and oviposition deterrence of leaf extracts of *Ipomoea cairica*
82 against dengue vectors [6]; ovicidal and repellent activity of several botanical extracts against
83 *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* [14]; and the ovicidal and
84 larvicidal activity of some plant extracts against *Cx. quinquefasciatus* and *Ae. aegypti* [13] have
85 been documented. The larvicidal, ovicidal and oviposition deterrent potential of neem oil water
86 dispersible tablets have been reported against *An. culicifacies* [15] while azadirachtin from neem
87 plant exhibited ovicidal activity against *Cx. tarsalis* and *Cx. quinquefasciatus* [16]. The larvicidal
88 and ovicidal activity of *Artocarpus blancoi* extracts were noted against *Ae. aegypti* [17] while the
89 larvicidal, ovicidal, and repellent activity of *Sophora alopecuroides* and synergistic activity of its
90 dominant constituents have been documented against *Ae. albopictus* [18].

91 Efforts to control malaria transmission in disease endemic areas are heavily reliant on suppression
92 of the vector populations through a combination of chemicals, biological methods and
93 management of breeding sites [19]. Consequently, application of adulticides and larvicides has
94 been a common strategy used in vector control. It is of essence that focus be also directed to the
95 egg stage in the mosquito development cycle due to its limited movement compared to the free
96 flying and swimming adult mosquitoes and larvae, respectively [20]. Consequently, discovery and
97 development of effective and environmental-friendly ovicidal compounds alongside the
98 identification and focus on most productive/viable breeding sites/ habitats for mosquito is crucial
99 for malaria vector and disease control [21].

100 2-Hydroxy-4-methoxybenzaldehyde (**1**), a structural isomer of vanillin (**2**), is an aromatic taste-
101 modifying compound commonly found in the root bark of *Mondia Whytei* plant [22]. It has been
102 previously reported as a tyrosine inhibitor [23] and potent larvicide against *An. gambiae* [24].
103 However, we could not find any information on its ovicidal activity or any structure-activity
104 relationship studies on it or related compounds against *An. gambiae* eggs in the literature.
105 Consequently, this project was designed to investigate the ovicidal activity of 2-hydroxy-4-
106 methoxybenzaldehyde, its derivatives, structural analogues and their blends on *An. gambiae* eggs
107 in order to understand the structure-ovicidal activity relationships therein.

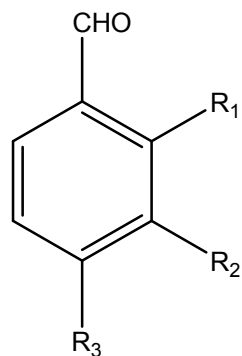
108 **Materials and methods**

109 **Insect culture**

110 The *An. gambiae* mosquitoes that produced eggs used in this study were reared under ambient
111 conditions of 27±1 °C and 85% relative humidity (RH), in the insectary situated at Centre for
112 Disease Control CDC at the Kenya Medical Research Institute, Kisumu, Kenya. They were fed
113 on 10% sucrose solution and a blood meal, to ensure that they produced viable eggs for the ovicidal
114 experiments.

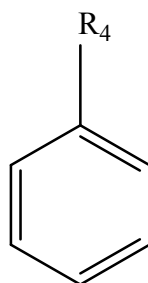
115 **Compounds for ovicidal assay**

116 2-Hydroxy-4-methoxybenzaldehyde (**1**) was isolated from *Mondia whytei* Skeels while
117 compounds **2-10** were procured from LOBA CHEMIE PVT LTD.



1. R₁=OH R₂=H R₃=OCH₃

2. R₁=H R₂=OCH₃ R₃=OH



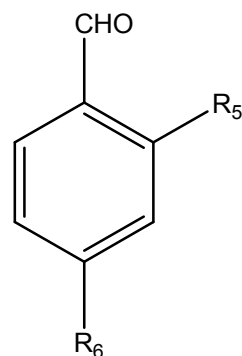
3. R₄=H

4. R₄=CHO

5. R₄=OH

6. R₄=OCH₃

7. R₄=COOH



8. R₅=OH R₆=H

9. R₅=H R₆=OCH₃

10. R₅=H R₆=OH

118

119 Compounds **11-13** were prepared in the laboratory as described below.

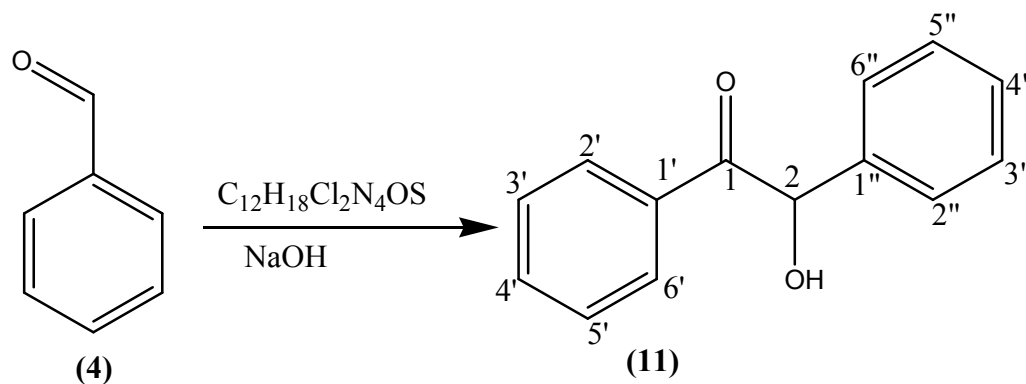
120 **Isolation of 2-hydroxy-4-methoxybenzaldehyde**

121 Fully developed roots were harvested from mature *Mondia whytei* plants cultivated at the Centre
122 for African Medicinal & Nutritional Fauna & Flora (CAMNFF) herbal garden at Masinde Muliro
123 University of Science & Technology, Kakamega, Kenya. They were cleaned with water and stored
124 under shade awaiting extraction. The roots were chopped into small pieces and subjected to
125 isolation using the established procedures [25]. The white crystalline compound [10 g, mp 41-43
126 °C] was obtained from 1000 g (10% yield) of the roots and confirmed to be 2-hydroxy-4-
127 methoxybenzaldehyde (**1**) from NMR and ESI-MS data [22]. It was stored in sealed amber bottles
128 and refrigerated at 4 °C awaiting ovicidal assays.

129 **2-Hydroxy-1, 2-diphenylethanone (11)**

130 The compound was prepared through established procedures summarized in Scheme 1 [26].

131 Scheme 1: Preparation of 2-hydroxy-1, 2-diphenylethanone (**11**)

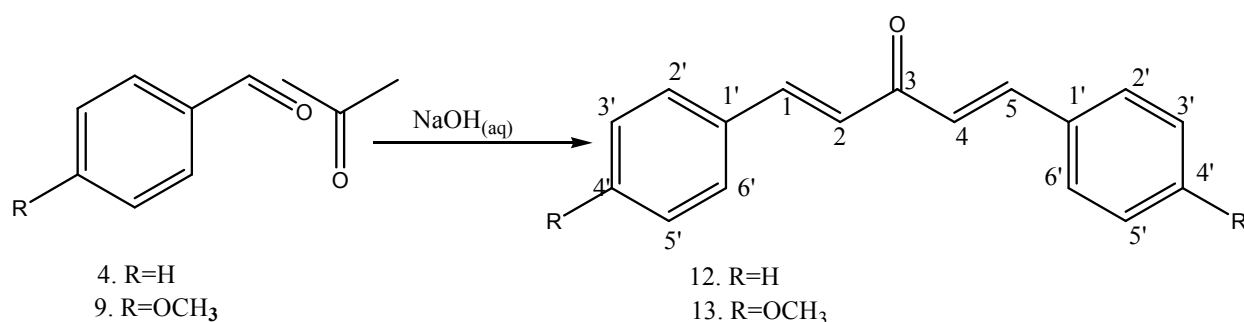


133 Briefly, thiamine hydrochloride (3 g, 8.04 mMol.) was dissolved in distilled water (4.5 mL) in a
134 250 mL conical flask, ethanol (30 mL) was added to the solution and the contents of the flask
135 swirled by hand for 15 min until homogeneity was achieved. Addition of NaOH (9 mL, 0.25 Mol.)
136 solution turned the mixture from colorless to bright yellow and the flask put on a mechanical shaker
137 at 300 rpm for 20 min until the bright yellow color changed to pale yellow. Benzaldehyde (9 mL,
138 9.5 g, 85 mMol.) was slowly added to the mixture and the flask loaded onto a mechanical shaker
139 at 300 rpm for 20 min until the mixture became homogeneous, the flask stoppered and left to stand
140 in the dark for 72 hrs. The yellow crystals (8.25 g, 38.9 mMol.) were re-crystallized from hot
141 ethanol to give white needle-like crystals of 2-hydroxy-1, 2-diphenylethanone (**11**) (7.92 g, 37.4
142 mMol., 95% yield) as confirmed by physical and spectroscopic data: mp 135-137 °C (literature
143 134-136 °C) [26]); ¹H NMR (400 MHz, CD₃SO) δ 7.82 (2H, d, J=5.16, H-2',H-6'), 7.46 (2H, t,
144 J=4.28, H-3', H-5'), 7.76 (1H, m, H-4'), 7.39 (2H, d, J=6.8, H-3'', H-5''), 7.25 (2H, m, J=7.8, H-2'',
145 H-6'') 7.06 (1H, d, J=5.08, H-4''); 5.92 (1H, d, H-2), 3.17 (1H, s, OH); ¹H-¹H COSY (CD₃SO) see
146 Figure 1; ¹³C NMR (δ, CD₃SO) 199.65 (C-1) 140.2 (C-1''), 135.22 (C-1'), 133.69 C-4'), 128.93
147 (C-2', C-6'), 128.17 (C-2', C-6') 129.3 (C-2'', C-6''), 129.06 (C-3'', C-5''), 127.73 (C-4''), 76.14 (C-
148 2) ; DEPT 135 (CD₃SO) 133.69 (C-4'H-4'), 128.17 (C-2'' & C -6''H-2'' & H-6''), 129.06 (C-3'' &
149 C-5''H-3'' & 5''), 128.93 (C-2' & C-6'H-2' & H-6'), 127.73 (C-4''H-4''), 128.93 (C-2' & C-6'H-2' &

150 6'), and 76.14 (C-2H-2); ¹J C-H, HSQC (CD₃SO) C-2' H-2', C6' H-6', , C-3' H-3' C-5'H-5' C-3"
151 H-3" C-5"H-5", C-4" H-4", C-2" H-2" C-6"H-6", C-4" H-4", C-2 H-2; ³J, ⁴J H-C HMBC (CD₃SO):
152 see Figure 2; EIMS (*m/z*) 39, 51, 63, 77, 105 (100%) [C₇H₅O]⁺, 139, 165 210 [M⁺].

153 Compounds **12** and **13** were prepared through the established procedures summarized in Scheme
154 2 [27].

155



157 **1, 5-Diphenylpenta-1, 4-diene-3-one (12)]]**

158 Briefly, 0.25 M NaOH solution (100 mL,) was transferred into a 500 mL conical flask, ethanol (80
159 mL) added and the mixture loaded onto a mechanical shaker at 200 rpm for 15 min. to attain
160 homogeneity. Acetone (4 mL, 3.16 g, 54.4 mMol.) and benzaldehyde (12 mL, 12.47 g, 117.5
161 mMol.) were added and shaken at 200 rpm for 20 min while the mixture changed from pale yellow
162 to deep yellow. On settling down, two layers were observed with the yellow crystals in the organic
163 layer. The layers were separated, the organic layer filtered using a suction pump, the crystals
164 collected, and dried to give 8.90 g. The crystals were carefully cleaned with methanol to obtain
165 shiny disk like yellow crystals of 1,5-diphenylpenta-1,4-diene-3-one (**12**) (8.74 g, 37.5 mMol.,
166 92% yield) and identified by physical and spectroscopic methods: mp 109-111 °C (literature 108-
167 110 °C) [27]; ¹H NMR (δ, CD₃OD) 7.61 (4H, d, J=16.18, H-2', H-6'), 7.32 (4H, d, J=8.04, H-3',

168 H-5'), 7.16 (2H, t, J=8.2, H-4'), 6.32 (2H, d, J=16.12, 12.64, H-2, H-4), 7.49 (2H, d, J=16.12, H-1,
169 H-5); ¹H-¹H COSY (CD₃OD): see Figure 3; ¹³C NMR (δ, CD₃OD) 190.11 (C-3), 143.8 (C-1, C-
170 5), 134.8 (C-1'), 130.38 (C-4'), 128.7 (C-2', C-6'), 128.3 (C-3', C-5'), 124.97 (C-2, C-4); DEPT 135
171 (δ) 143.8(C-1H-1,C-5 H-5) 130.38 (C-4'H-4'), 128.7 (C-2'H-2',C-6' H-6'), 128.3 (C-3'H-'), 124.97
172 (C-2 H-2, C-4 H-4); ³J, ⁴J H-C HMBC: see Figure 4; and EIMS (*m/z*) 39, 51, 63, 77, 91, 103,
173 115, 131, 156, 191, 205, 215,., 233 (100%) [M⁺-H], 234 [M⁺]

174 **1, 5-Bis (4-methoxyphenyl) penta-1, 4-diene-3-one (13)**

175 Briefly, 0.25 M NaOH solution (25 mL) was poured into a 100 mL conical flask, 10 mL of ethanol
176 added, and the mixture shaken at 200 rpm for 15 min. to attain homogeneity after which acetone
177 (1.4 mL, 1.11 g, 19 mMol.) and 4-methoxybenzaldehyde (5.3 mL, 5.51 g, 40 mMol.) were
178 sequentially added to form a white emulsion. The emulsion was shaken at 200 rpm for 10 min.,
179 allowed to settle down and form two layers: pale yellow and deep yellow, with fat-like droplets on
180 top of the deep yellow layer, which changed into yellow crystals after 30 min. The mixture was
181 filtered using a suction filtration pump to afford yellow crystals which were dried and cleaned
182 carefully with methanol to give shiny disk-like yellow crystals of 1.5-bis-(4-
183 methoxyphenyl)penta-1,4-diene-3-one (**13**) (3.83g, 13 mMol, 85% yield): mp 105-107 °C
184 (literature 105-107 °C) [28]; ¹H NMR (δ CDCl₃) 7.89 (4H, d, J=8.8, H-2', H-5',), 7.65 (4H, d,
185 J=8.8, H-3', H-5'), 7.42 (2H, d, J=15.8, H-1, H-5), 6.99 (2H, d, J=15.8, H-2 H-4). 3.87 (3H, s,
186 OCH₃); ¹H-¹H COSY (CDCl₃): see Figure 5; ¹³C NMR (δ, CDCl₃) 188.86 C-3) 161.57 (C-4'),
187 142.67 (C-1, C-5), 130.07 (C-2', C-5'), 127.68 (C-1'), 123.54 (C-2, C-4), 114.43 (C-3', C-5'), 55.41
188 (OCH₃); DEPT 135 (δ, CDCl₃) 142.67(C-1H-5), 130.07 (C-2'H-2'), 130.07 (C-5', H-5'), 123.54
189 (C-2H-2), 114.43 (C-3',H-3'), 114.43 (C-5'H-5'), 55.41 (OCH₃); ¹J C-H, HSQC (δ, CDCl₃) C-2

190 H-2 4C-4 H-4, C-1 H-1 C-5H-5, C-3' H-3', C-5'H-5', C-2' H-2'; ³J, ⁴J H-C HMBC: see Figure 6;
191 EIMS (*m/z*)[31, 39, 41, 53, 56, 57, 59 (100%) [C₃H₄O]⁺, 63, 73, 83, 87, 100, 294 [M⁺].

192 **Preparation of stock solution and dilutions**

193 The pure compounds (10 mg) were dissolved in 10 mL of ethanol and topped up to 100 mL with
194 distilled water to prepare 100 ppm stock solutions. The stock solutions were diluted appropriately
195 with distilled water to obtain 1, 10, 25 and 50 ppm solutions for ovicidal assays.

196 Blends were formulated from benzaldehyde (**4**) (B), phenol (**5**) (P) and anisole (**6**) (A). Briefly,
197 the individual compounds (3.33 mg each) were mixed to form blend BPA, which was dissolved in
198 ethanol (10 mL) and topped up to 100 mL of distilled water to make a stock solution of 100 ppm.
199 For blends PA, BP and BA, equal amounts of individual compounds (5 mg) were mixed and
200 dissolved in 10 mL of ethanol and topped up to 100 mL with distilled water to make 100 ppm
201 stock solutions. The stock solutions were diluted appropriately with distilled water to make 1, 2,
202 10, 25 and 50 ppm for ovicidal assays.

203 **Ovicidal activity**

204 Ovicidal activity was determined by measuring the inhibition of egg hatchability. Briefly, freshly
205 laid eggs of *An. gambiae* were counted and divided into groups of 100 using a hand magnifying
206 lens and each group submerged into 25 mL of 1, 2, 10, 25 and 50 ppm solutions of each pure
207 compounds in transparent plastic containers for 48 hours or until they hatched into larvae or
208 completely inhibited from hatching. Each treatment was replicated 4 times, with eggs exposed to
209 1% ethanol in water and plain distilled water serving as controls. The hatchability was assessed
210 after 48 hours of post treatment. The emergent larvae were also observed for survival rate and
211 deformities.

212 **Statistical analysis**

213 The ovicidal data were compared using Students Newman Kuel *t*-tests (SNK *t*-test) and the dose-
214 response-relationships determined using probit analysis. The LD₅₀ and LD₉₀ values obtained from
215 the regression analysis [29]. The level of significance of statistical data was set at $p < 0.05$ or
216 lower.

217 **Results and discussion**

218 Thirteen compounds (**1-13**) grouped into five molecular structures (**1-2, 3-7, 8-10, 11, 12-13**) were
219 tested individually and as blends for ovicidal activity using the eggs of *Anopheles gambiae*
220 mosquitoes.

221 The results in table 1 indicate the hatchability rate of the *An. gambiae* eggs in different treatments
222 at various concentrations. Highest egg mortality or un-hatchability was observed at 50 ppm in
223 nearly all the compounds tested. Poor ovicidal activity was noted for all the treatments at 1 ppm
224 since almost all of the *An. gambiae* eggs hatched into larvae. The larvae that emerged had no
225 significant deformities observed.

226 **Structure activity relationship**

227 2-Hydroxy-4-methoxybenzaldehyde (**1**), a trisubstituted aromatic compound with hydroxyl,
228 methoxyl and aldehydic groups, has been previously isolated from the roots of *Mondia whytei*,
229 shown to be as a tyrosine inhibitor [23] and a potential larvicide for *An. gambiae* [24]. The
230 compound is also responsible for the characteristic smell and taste of roots from the plant [22]. It
231 is the reported insecticidal properties and the diverse functional groups that prompted us to probe
232 its ovicidal activity against *An. gambiae* eggs to establish whether it has potential to inhibit the

233 eggs from hatching and if so which functional groups confer the activity. The ovicidal activity of
234 2-hydroxy-4-methoxybenzaldehyde (**1**), related compounds (**2-10**), structural analogues (**11-13**)
235 and formulated blends against *An. gambiae* are summarized in table 2. With an LD₅₀ value of
236 0.7075 ppm, compound **1** prompted a structure activity relationship to probe the functional groups
237 responsible for the observed ovicidal efficacy. Readily available and a closely related congener; 4-
238 hydroxy-3-methoxybenzaldehyde/ vanillin (**2**) was bioassayed and the activity found to be 34
239 times lower (LD₅₀ 24.177 ppm) than **1**). Interestingly, lower tyrosinase inhibition and larvicidal
240 activity of vanillin (**2**) and other related congeners have also been reported [23-24]. The big
241 difference in the biological activity of the two compounds is quite intriguing given that they have
242 similar functional groups save for their relative positions to each other on the aromatic skeleton.
243 This observation prompted further investigations on the cause of varied activity in regard to the
244 observed ovicidal activity. Consequently, related compounds with similar functional groups but
245 different functional group arrangement on the benzene skeleton were assayed for comparison.

246 Simple aromatic compounds constituting similar functional groups like those on **1** and **2** were
247 assayed. Anisole or methoxybenzene (**6**) (LD₅₀ 40.342 ppm), benzoic acid (**7**) (LD₅₀ 25.633 ppm)
248 and benzene (**3**) (LD₅₀ 19.494 ppm) exhibited low activity while benzaldehyde (**4**) at LD₅₀ 5.584
249 ppm had slightly higher activity than phenol (**5**) (LD₅₀ 9.9354 ppm). The results revealed an
250 interesting trend in the potency of derivatives of benzene (**3**) due to substituent variation that
251 enhance or lower activity of the resulting aromatic compound. Further, they demonstrate that the
252 aldehyde functional group is more potent when attached to the benzene ring than carboxylic acid
253 as in compound **7** that resulted in much lower ovicidal activity than **4**. This observation is
254 consistent with earlier reports where 4-methoxysalicylic acid was found to exhibit lower
255 larvicidal activity than 2-hydroxy-4-methoxybenzaldehyde [24]. The enhanced ovicidal activity

256 of compounds **4** and **5** suggest that attachment of an aldehyde or hydroxyl group on **3** enhances its
257 efficacy. On the hand, methoxyl and carboxylic acid groups on benzene ring lowers the ovicidal
258 activity of the resultant compound drastically. While the bioassay data of these simple compounds
259 were supposed to help us understand the individual contribution of the individual functional groups
260 on the activity of 2-hydroxy-4-methoxybenzaldehyde (**1**) and vanillin (**2**), they could not
261 satisfactorily explain the observed activity of compounds **1** and **2**, and therefore more assays using
262 di-substituted aromatic compounds were undertaken to investigate the structure-activity
263 relationships in the two compounds. Due to relatively strong ovicidal activity, benzaldehyde (**4**)
264 was chosen as the starting point for the structure-activity studies. The introduction of an electron
265 donating hydroxyl group at *ortho* position in compound **4** resulted in 2-hydroxybenzaldehyde (**8**)
266 with improved ovicidal activity (LD₅₀ 1.452 ppm), confirming that *ortho*-hydroxyl group
267 synergizes ovicidal activity of benzaldehyde. However, when the hydroxyl group was shifted to
268 *para* position as in 4-hydroxybenzaldehyde (**10**), the activity was drastically lowered to LD₅₀
269 15.642 ppm, confirming that *para*-hydroxyl group is antagonistic to the ovicidal activity of
270 benzaldehyde. The two observations unravel the contribution of the hydroxyl group on the activity
271 of **1** and **2** and confirm that the relative position of the substituents on the benzene skeleton is
272 critical. Interestingly, addition of a stronger electron donating methoxy group at the *para* position
273 of **4** gives 4-methoxybenzaldehyde (**9**) with increased activity at LD₅₀ of 1.390 ppm. The activity
274 of compound **9** helped us to explain the low ovicidal activity observed in vanillin (**2**), a tri-
275 substituted aromatic compound. Considering the observed low activity of 4-hydroxybenzaldehyde
276 (**10**), addition of methoxy group *ortho* to the hydroxy and *meta* to the carbonyl gives vanillin (**2**)
277 with lower activity. It is interesting to note that shifting the hydroxyl of 2-hydroxybenzaldehyde
278 (**8**) to *para* lowers the activity of the resulting 4-hydroxybenzaldehyde (**10**), further addition of

279 methoxyl *ortho* to the hydroxyl of 4-hydroxybenzaldehyde (**10**), gives vanillin (**2**) with much
280 lower activity than 4-hydroxybenzaldehyde (**10**). The trend in the activity of vanillin (**2**), 2-
281 hydroxybenzaldehyde (**8**) and 4-hydroxybenzaldehyde (**10**) assisted in assessing the effect of
282 substituent position on the aromatic ring. It further demonstrates that the hydroxyl and methoxyl
283 groups are either synergistic or antagonistic to the ovicidal effect of the carbonyl when on the same
284 ring depending on the position of attachment. In addition, the hydroxyl and methoxyl groups are
285 inactive when *ortho* relative to each other as earlier reported for larvicidal activity [24]. On the
286 contrary, 2-hydroxy-4-methoxybenzaldehyde (**1**) displays quite an interesting trend in activity.
287 Considering 2-hydroxybenzaldehyde (**8**) and 4-methoxybenzaldehyde (**9**), the addition of methoxy
288 at *para* to the carbonyl and *meta* to the hydroxyl of **8** increases the activity of **1**. Similarly, the
289 addition of hydroxyl *ortho* to the carbonyl and *meta* to the methoxyl group in 4-
290 methoxybenzaldehyde (**9**) gives 2-hydroxy-4-methoxybenzaldehyde (**1**) with increased activity
291 suggesting that methoxyl and hydroxyl groups are potentiating the benzaldehyde group depending
292 on their positions relative to the carbonyl and to each other when all the three functional groups
293 are on the same benzene ring

294 The electron donating hydroxyl and methoxyl groups gave interesting results when attached at
295 *para* to aldehyde carbonyl. It is important to note the fundamental role played by the slightly
296 bulky methoxyl in increasing activity as in 4-methoxybenzaldehyde (**9**); while on the other hand,
297 the hydroxyl group in 4-hydroxybenzaldehyde (**10**) lowered activity. The free *para*-hydroxyl group
298 plays more antagonistic ovicidal role to the aldehydic carbonyl position than when it is at *ortho*
299 position probably due to stronger inter-molecular H-bonding than the intra-molecular ones in **8**
300 which enhance activity. These observations can also be rationalized by the stronger electron
301 donating property of *para*-methoxy than *para* hydroxyl group and the intra-molecular hydrogen

302 bonding to the carbonyl by the *ortho*-hydroxyl group. The enhanced activity of compounds **8**, **9**
303 and **10** therefore reflects the effectiveness of the position of hydroxyl and methoxyl groups in
304 relation to aldehyde group as demonstrated in compounds **1** and **2** where it was noted that the
305 position of methoxyl or hydroxyl has impact on activity.

306 Compound **4** was modified to **11** and **12** while compound **9** gave **13**. The structural analogs were
307 assayed for ovicidal activity. Compounds **11** (LD₅₀ 10.599 ppm) and **12** (LD₅₀ 9.019 ppm)
308 exhibited lower activity exhibited lower ovicidal activity than the parent compound **4**. Similarly,
309 compound **13** exhibited lower activity (LD₅₀ 15.642ppm) than the parent compound **9**. The
310 observations suggest that the aldehyde and hydroxyl groups are critical for ovicidal activity of *An.*
311 *gambiae* eggs as previously reported for larvicidal activity [24].

312 The blends assayed for ovicidal activity included benzaldehyde (**4**), phenol (**5**), and anisole (**6**)
313 (**BPA**); (benzaldehyde and anisole) (**BA**), (benzaldehyde and phenol) (**BP**) and (phenol and
314 anisole) (**PA**). **BPA**, a blend of compounds **4**, **5** & **6** exhibited better ovicidal activity at LD₅₀
315 2.944 ppm than any of the individual components, but was four times lower than 2-hydroxy-4-
316 methoxybenzaldehyde (**1**) and eight times higher than vanillin (**2**), indicating that the compounds
317 exert synergy when in the blend. Blend **PA**, equivalent to subtraction of compound **4** from **BPA**,
318 lowered its activity by almost half to LD₅₀ 5.129 ppm thus indicating that benzaldehyde is a critical
319 component of the blend. Blend **BP**, equivalent to substituting compound **6** with **4** or subtracting
320 **6** from blend **BPA**, exhibited the highest ovicidal activity LD₅₀ 0.332 ppm which was nine (9)
321 times higher than that of blend **BPA** and fifteen (15) times higher than that of blend **PA** and
322 confirmed that benzaldehyde is a critical component of the blend. Blend **BA**, equivalent to the
323 subtraction of compound **5** from **BPA** or substitution of compound **5** from **BP**, exhibited the lowest

324 ovicidal activity of all the blends at LD₅₀ 9.990 ppm confirming that anisole is an antagonistic
325 component of the blend. The observed results revealed that the synergistic interaction of the
326 individual compounds is much stronger when the compounds are blended than when all the
327 functional groups are incorporated in one compound suggesting that intra-molecular interactions
328 have higher positive impact on ovicidal activity than the inter-molecular interactions.

329 Several structure-larvicidal activity relationships have been documented with all the studies
330 linking functional groups of different compounds to the resulting activity of the compounds. For
331 instance, acetyl derivatives of monoterpenoid compounds were reported to have high activity
332 against the larvae of *Ae. aegypti* [30]. In another case, presence of hetero-atoms in the basic
333 monoterpene structure for instance neoisopulegol reduced the potency of the compound. It was
334 further noted that conjugated or *exo*-carbon-carbon double bonds and epoxidation increased
335 larvicidal activity [31]. The larvicidal assay of eugenol and its derivatives revealed that the
336 derivatives had lower activity [32]. Furthermore, it has been reported that conversion of phenol to
337 diphenyl ether increased the activity against *An. gambiae* larvae [24].

338 **Conclusion**

339 Finally our work established that the hydroxyl, methoxyl and aldehyde functional groups on an
340 aromatic skeleton confer ovicidal activity when appropriately located in one compound but are
341 strongly synergistic when in different molecules. 2-Hydroxy-4-methoxybenzaldehyde exhibited
342 the highest ovicidal activity against *An. gambiae*, while anisole exhibited the lowest efficacy.
343 Simple mono-functional compounds: benzaldehyde, phenol and anisole exhibited relatively low
344 activity than when evaluated individually than when formulated as blends. Among the blends,
345 blend **BP** exhibited the highest activity, while **BA** had the lowest efficacy. The presence of
346 aldehyde and hydroxyl groups on mono-substituted benzene confers strong ovicidal activity while

347 methoxyl group lowers activity. For di-substituted simple aromatic compounds, methoxyl group
348 is an activity-potentiating group at *para* position to the aldehyde group and hydroxy when in ortho-
349 position to the aldehyde.

350 **Acknowledgements**

351 This work was supported by Masinde Muliro University of Science and Technology (MMUST)
352 University Research Fund (URF). We appreciate Mr. Richard Amito and his team at the Kenya
353 Medical Research Institute (KEMRI) for the supply of *An. gambiae* eggs and the help accorded to
354 us during the bioassays. Center for African Medicinal and Nutritional Flora and Fauna (CAMNFF)
355 is acknowledged for provision of *Mondia whitei* roots and the laboratory space for synthetic work.

356

357 **References**

- 358 1. WHO (2010). World Malaria Report, Geneva: World Health Organization.
- 359 2. WHO (2020). World Malaria Report: 20 Years of Global Progress and Challenges: World
360 Health Organization, Geneva; Licence: CC BY-NC-SA 3.0 IGO.
- 361 3. Senthil N. (2009). The use of *Eucalyptus tereticornis* Sm.(Myrtaceae) oil (leaf extract) as a
362 natural larvicidal agent against the malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae).
363 *Bioresource Technology* 98: 1856-1860.
- 364 4. Takken W, Knols BGJ (1999). Odour mediated behaviour of afro-tropical malaria mosquitoes.
365 *Ann. Rev. Entomol.* 44: 131-57.

- 366 5. WHO (1995). Vector Control for Malaria and Other Mosquito-borne Diseases. Report of a
367 WHO Study Group. WHO Technical Report Series 857, World Health Organization, Geneva,
368 Switzerland.
- 369 6. Ahbirami R., Zuharah W.F., Yahaya Z.S., Dieng H., Thiagaletchumi M, Fadzly N., *et al.* (2014)
370 Oviposition deterring and oviciding potentials of *Ipomoea cairica* L. leaf extract against dengue
371 vectors. *Tropical Biomedicine* 31: 456–465.
- 372 7. Bowers WS. (1992) Biorational approaches for insect control. *Korean J. Appl. Entomol.* 31:
373 289-303.
- 374 8. Ghosh .A, Chowdhury N., Chandra G. (2008). Laboratory evaluation of a phytosteroid
375 compound of mature leaves of Day Jasmine (Solanaceae: Solanales) against larvae of *Culex*
376 *quinquefasciatus* (Diptera: Culicidae) and non-target organisms. *Parasitology Research.* 103: 271-
377 277.
- 378 9. Sutthanont N., Choochote W., Tuetun B., Junkum A., Jitpakdi A., Chaithong U, *et al.* (2010).
379 Chemical composition and larvicidal activity of edible plant-derived essential oils against the
380 pyrethroid-susceptible and resistant strains of *Aedes aegypti* (Diptera: Culicidae). *J. Vector Ecol.*
381 35: 106-115.
- 382 10. Bayen S. (2012) Occurrence, bioavailability and toxic effects of trace metals and organic
383 contaminants in mangrove ecosystems: a review. *Environment International* 48: 84-101.
- 384 11. Mulyatno KC, Yamanaka A, Ngadino, Konishi E. (2012). Resistance of *Aedes aegypti* (L.)
385 larvae to temephos in Surabaya, Indonesia. *S. E. Asian J.Trop. Med Pub. Health* 43: 29-33.

- 386 12. Chavshin AR, Dabiri F, Vatandoost H, Bavani MM. (2015) Susceptibility of *Anopheles*
387 *maculipennis* to different classes of insecticides in West Azarbaijan Province, Northwestern Iran,
388 *Asian Pacif. J. Trop. Biomed.* 5: 403-6.
- 389 13. Munusamy R., Appadurai D.R., Kuppusamy S., Michael G.P., Savarimuthu I. (2016).
390 Ovicidal and larvicidal activities of some plant extracts against *Aedes aegypti* L. and *Culex*
391 *quinquefasciatus* Say (Diptera: Culicidae). *Asian Pacif. J. Trop. Disease* 6: 468-471.
- 392 14. Govindarajan M., Mathivanan T., Elumalai K., Krishnappa K., Anandan A. (2011) Ovicidal
393 and repellent activities of botanical extracts against *Culex quinquefasciatus*, *Aedes aegypti*, and
394 *Anopheles stephensi*. (Diptera:Culicidae). *Asian Pac. J. Trop. Biomed.* 1: 43-48.
- 395 15. Kala S., Naika S.N., Patanjali P.K., Sogan N. (2019). Neem oil water dispersible tablet as
396 effective larvicide, ovicide and oviposition deterrent against *Anopheles culicifacies*. *S. Afr. J.Bot.*
397 123: 387-392.
- 398 16. Su T., Mulla S. (1998) Ovicidal activity of neem product (azadirachtin) against *Culex tarsalis*
399 and *Culex quinquefasciatus* (Diptera: Culicidae). *J.Amer. Mosquito Control Assoc.* 14: 204-209.
- 400 17. Pineda-Cortel M.R.B., Cabantog R.J.R., Caasi P.M., Ching C.A.D., Perez J.B.S., Godisan
401 P.G.M., *et al.* (2019). Larvicidal and ovicidal activities of *Artocarpus blancoi* extracts against
402 *Aedes aegypti*. *Pharmaceutical Biology* 57:, 120-124; DOI:10.1080/13880209.2018.1561727.
- 403 18. Shoukat R.F., Shakeel M., Rizvi S.A.H., . Zhang J.Z.Y, . Freed S., X. Xu *et al.* (2020)
404 Larvicidal, ovicidal, synergistic, and repellent activities of *Sophora alopecuroides* and its
405 dominant constituents against *Aedes albopictus*. *Insects* 11: 246-259;
406 DOI:10.3390/insects11040246.

- 407 19. WHO (2009) Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control, New
408 edition. World Health Organization, Geneva.
- 409 20. Elimam A.M., Elmalik K.H., Ali F.S. (2009). Larvicidal, adult emergence inhibition and
410 oviposition deterrent effects of foliage extract from *Ricinus communis* L. against *Anopheles*
411 *arabiensis* and *Culex quinquefasciatus* in Sudan. *Tropical Biomedicine* 26: 130-139.
- 412 21. WHO (1996) Report of the Consultation on key Issues in dengue vector control towards the
413 operationalization of a global strategy. World Health Organisation, Geneva.
- 414 22. Mukonyi K.W., Ndiege I.O. (2001) 2-Hydroxy 4-methoxybenzaldehyde: aromatic taste
415 modifying compound from *Mondia whitei* Skeels. *Bull. Chem. Soc. Ethiopia* 15:137–141.
- 416 23. Kubo I., Kinst-Hori I., (1999). 2-Hydroxy-4-methoxybenzaldehyde: a potent tyrosinase
417 inhibitor from African medicinal plants. *Planta Medica* 65: 19-22.
- 418 24. Mahanga G.M., Akenga T.O., Lwande W., Ndiege I. O. (2005). 2-hydroxy-4-
419 methoxybenzaldehyde: larvicidal structure activity studies. *Bull. Chem. Soc. Ethiopia* 61-68.
- 420 25. Rathi N., Keerthana H., Jayashree V., Sharma A. & Rao N.N. (2017) 2-Hydroxy-4-
421 methoxybenzaldehyde, an astounding food flavoring metabolite: A review. *Asian J. Pharmaceutic.*
422 *Clin. Res.* 10: 105-110.
- 423 26. Rajeev S. M., Akkattu T. B., Vijay N. (2016). Recent advances in N-heterocyclic carbene
424 (NHC)-catalyzed benzoin reactions. *Beilstein J. Org. Chem.* 12: 444-461, DOI:
425 10.3762/bjoc.12.47.
- 426 27. Nagaraja N., Vijay H. K, Swetha S. (2011). 1, 5-Diphenylpenta-1, 4-dien-3-ones: A novel
427 class of free radical scavengers. *Bulgarian Chem. Comm.* 43: 460-464.

- 428 28. Harrison W. T. A., Sarojini B. K., Vijaya R. K. K., Yathirajan H. S., Narayana B. (2006). A
429 redetermination of 1,5-bis(4-methoxyphenyl)penta-1,4-dien-3-one at 120 (2) K. *Acta Cryst.*, E62,
430 [1522–1523]; DOI: 10.1107/S1600536806009640
- 431 29. SAS (2012) SAS Guide for Personal Computers, Version 8.2. SAS University Edition.
- 432 30. Pandey S. K, Tandon S., Ahmad A., Singh A. K., Tripathi A.K. *et al.* (2013). Structure-activity
433 relationships of monoterpenes and acetyl derivatives against *Aedes aegypti* (Diptera: Culicidae)
434 larvae. *Pest Manage. Sci.* 69: 1235–123; DOI:10.1002/ps.3488.
- 435 31. Sandra R.L., Melo M.A., Valenca A., Santos R.L.C., De Sousa D.P., Socates C.H. (2011)
436 Structure-Activity relationship of larvicidal monoterpenes and derivatives against *Aedes aegypti*
437 Linn. *Chemosphere* 84:150-153; DOI: 10.1016/j.chemosphere.2011.02.018.
- 438 32. Barbosa J.D.F., Silva V., Alves P., Giuseppe G., Roseli L.C., Damiao P. *et al.* (2012).
439 Structure-activity relationships of eugenol derivatives against *Aedes aegypti*. *Pest manage Science*
440 68(11):1478-83; DOI: 10.1002/ps.3331.

441

442

443

444

445

446

447

448 **Table 1: Means (Mean \pm SD) Number of Hatched Eggs of *An. gambiae* from Treatment with**
 449 **Compounds and Blends on at Various Concentrations**

Compound/Blend	Concentration			
	1 ppm	10 ppm	25 ppm	50 ppm
1	44.33 \pm 5.93 ^c	0.0 \pm 0.0 ^h	0.0 \pm 0.0 ^h	0.0 \pm 0.0 ^h
2	75.00 \pm 8.90 ^a	83.25 \pm 7.05 ^a	68.75 \pm 4.89 ^a	0.0 \pm 0.0 ^h
3	67.50 \pm 1.85 ^a	83.25 \pm 7.05 ^a	48.25 \pm 5.54 ^c	0.0 \pm 0.0 ^h
4	59.00 \pm 3.74 ^b	69.75 \pm 3.15 ^a	0.0 \pm 0.0 ^h	0.0 \pm 0.0 ^h
5	84.25 \pm 3.86 ^a	45.00 \pm 3.35 ^a	2.00 \pm 1.23 ^g	0.0 \pm 0.0 ^h
6	92.00 \pm 2.38 ^a	54.00 \pm 3.54 ^a	49.25 \pm 1.93 ^c	0.0 \pm 0.0 ^h
7	80.25 \pm 8.35 ^a	66.50 \pm 5.27 ^a	76.00 \pm 6.34 ^a	0.0 \pm 0.0 ^h
8	69.75 \pm 4.50 ^a	0.0 \pm 0.0 ^h	0.0 \pm 0.0 ^h	0.0 \pm 0.0 ^h
9	62.67 \pm 17.84 ^a	0.0 \pm 0.0 ^h	0.0 \pm 0.0 ^h	0.0 \pm 0.0 ^h
10	85.75 \pm 3.50 ^a	75.00 \pm 2.58 ^a	19.50 \pm 3.88 ^e	0.0 \pm 0.0 ^h
11	67.00 \pm 7.94 ^a	57.50 \pm 3.71 ^b	19.00 \pm 3.49 ^e	0.75 \pm 0.75 ^g
12	76.00 \pm 9.44 ^a	55.75 \pm 8.43 ^b	0.75 \pm 0.48 ^g	0.0 \pm 0.0 ^h
13	85.00 \pm 3.50 ^a	75.00 \pm 2.58 ^a	19.00 \pm 3.88 ^e	0.0 \pm 0.0 ^h
BPA	57.67 \pm 16.19 ^a	25.00 \pm 11.00	4.67 \pm 3.71 ^g	0.0 \pm 0.0
BA	75.33 \pm 6.74 ^a	69.00 \pm 3.605 ^a	0.0 \pm 0.0	0.0 \pm 0.0
BP	45.67 \pm 4.91 ^c	16.33 \pm 10.398 ^{e e}	0.0 \pm 0.0	0.0 \pm 0.0
PA	56.33 \pm 23.82 ^a	41.67 \pm 3.180 ^d	14.33 \pm 2.03 ^f	0.0 \pm 0.0
Ethanol	71.25 \pm 4.96 ^a	73.00 \pm 1.958 ^a	78.75 \pm 3.01 ^a	57.50 \pm 6.86 ^b

water	83.00±4.55 ^a			
-------	-------------------------	--	--	--

450 Means with the same letter are not significantly different (SNK test, p=0.0001).

451

452

453

454

455

456

457

458

459

460

461

462

463

464

465

466

467 **Table 2: Ovicidal Activity (LC₅₀ and LC₉₀) of Evaluated Compounds and Blends on *An.***
468 *gambiae* eggs

Compound/Blend	LC₅₀	LC₉₀
1	0.7075	4.5928
2	24.177	48.971
3	19.494	48.087
4	5.584	19.844
5	9.9354	20.289
6	40.342	100.894
7	25.633	49.830
8	1.4516	2.5707
9	1.3899	2.9366
10	15.642	30.626
11	10.599	32.582
12	9.019	20.252
13	15.642	30.626
BPA	2.944	19.047
BA	5.129	27.661
BP	0.3320	11.9848
PA	9.9909	20.8138

469

470 Captions

471 S1 Table 1: Means (Mean \pm SD) Number of Hatched Eggs of *An. gambiae* from Treatment with
472 Compounds and Blends on at Various Concentrations

473 S2 Table 2: Ovicidal Activity (LC₅₀ and LC₉₀) of Evaluated Compounds and Blends on *An.*
474 *gambiae* eggs

475 S3 Figure 1: ¹H-¹H COSY data for 2-Hydroxy-1, 2-diphenylethanone (**11**)

476 S4 Figure 2: ³J, ⁴J H-C HMBC data for 2-Hydroxy-1, 2-diphenylethanone (**11**)

477 S5 Figure 3: ¹H-¹H COSY data for compound **12** and **13**

478 Figure 3: ¹H-¹H COSY data for compound **12** and **13**

479 S6 Figure 4: ³J, ⁴J H-C HMBC data for compound **12** & **13**

480

481

482

bioRxiv preprint doi: <https://doi.org/10.1101/2021.09.14.460396>; this version posted September 14, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

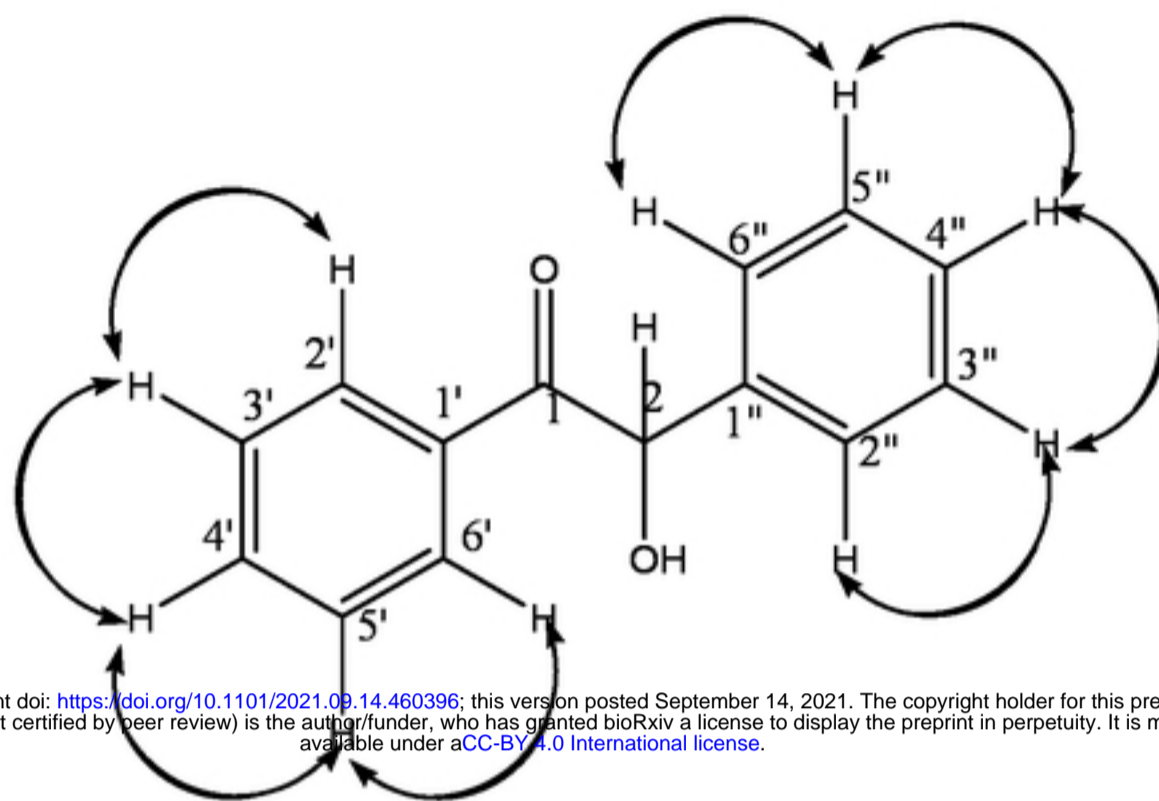
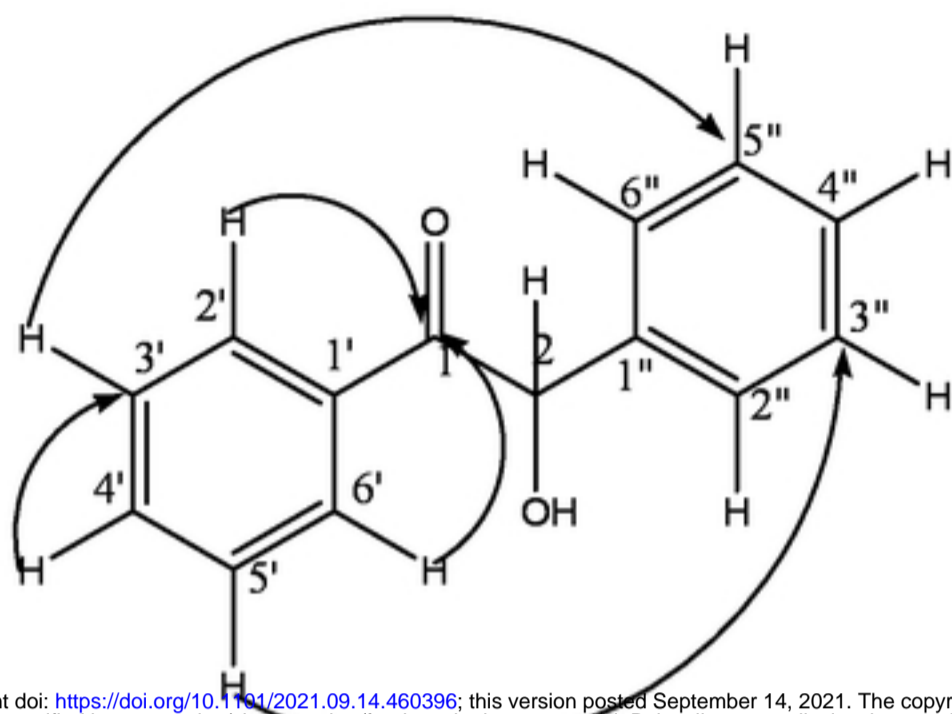
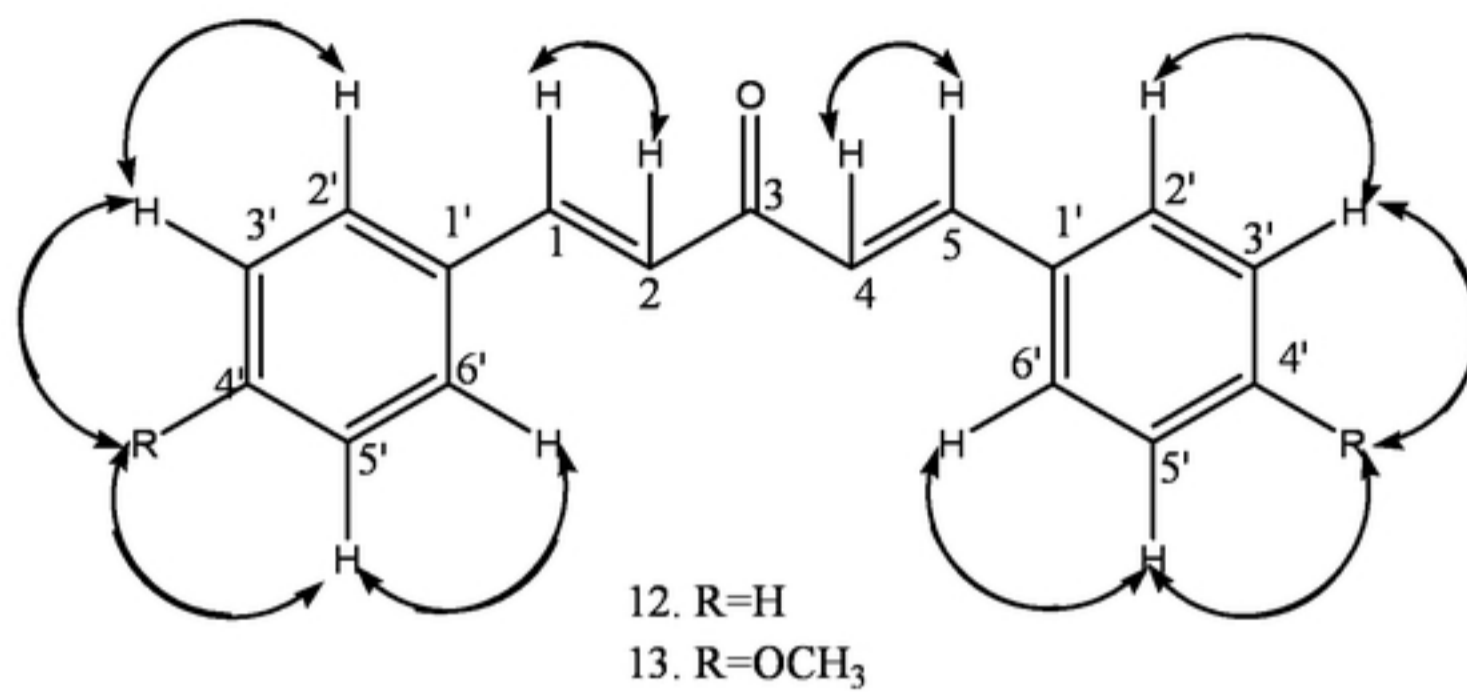


Figure 1: ^1H - ^1H COSY data for 2-Hydroxy-1,2-diphenylethanone (11)



bioRxiv preprint doi: <https://doi.org/10.1101/2021.09.14.460396>; this version posted September 14, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

Figure 2: 3J , 4J H-C HMBC data for 2-Hydroxy-1, 2-diphenylethaneone (**11**)



bioRxiv preprint doi: <https://doi.org/10.1101/2021.09.14.460396>; this version posted September 14, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

Figure 3: ¹H-¹H COSY data for compound 12 and 13

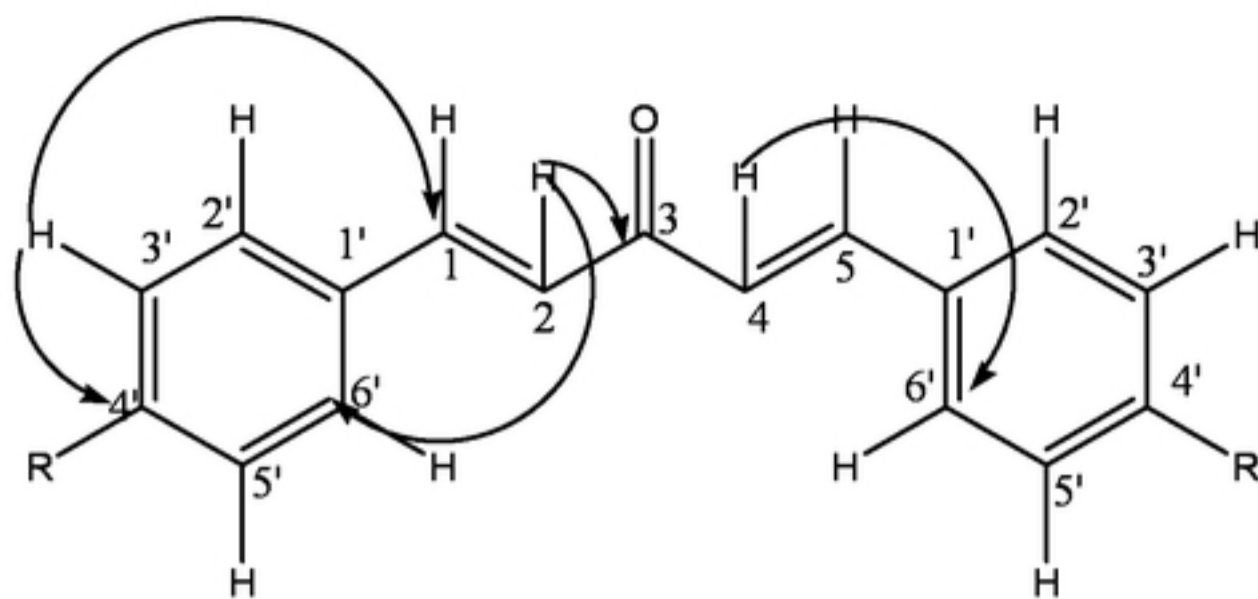


Figure 4: ^{31}P , $^4\text{J H-C}$ HMBC data for compound 12 & 13

bioRxiv preprint doi: <https://doi.org/10.1101/2021.09.14.460390>; this version posted September 14, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under a [CC-BY 4.0 International license](https://creativecommons.org/licenses/by/4.0/).