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Antibacterial Activity of Phytoalexins from Infected *Theobroma cacao* L

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Authors' contributions

This work was carried out in collaboration between all authors. Authors EDF, BIA and SAA designed the study and wrote the protocol. Author EDF carried out the microbiological analysis. Authors EDF and BIA analysed and interpreted the result while Author SAA read and interpreted the spectra data. Author EDF managed the literature search and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To study the antimicrobial activities of the compounds produced due to reactions of cocoa (*Theobroma cacao*) with *Phytophthora palmivora* during infection.
Place and Duration of Study: Department of Microbiology, Ekiti State University, Ado Ekiti, Nigeria and the Department of Pharmacognosy, Obafemi Awolowo University, Ile-Ife, Nigeria between September, 2009 and July, 2011.
Methodology: *Phytophthora palmivora* was used to infect healthy cocoa pods. The phytoalexins were extracted using solvent extraction and purified using standard methods. Agar diffusion and paper disc methods were used to study the antibacterial activities of the compounds extracted.
Results: Two major compounds were subsequently isolated, purified and characterized as FC-3-B21 and FC-4-B22 using the ^{1}HNMR and ^{13}CNMR as well as the 2D cosy data. Compound FC-3-

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B21 was characterized as 7,8,9,-trihydroxy-2,8-dihydroxy naphtha-10-one while FC-4-B22 was characterized as ester of glycerol: *Bacillus subtilis, Escherichia coli, Micrococcus luteus, Pseudomonas aeruginosa* and *Staphylococcus aureus,* were all sensitive to 7,8,9,-trihydroxy-2,8-dihydroxy naphtha-10-one even at 20 mg/ml. *Pseudomonas aeruginosa* showed the least sensitivity with 2.0 mm zone of inhibition at 20 mg/ml and 12 mm at 100 mg/ml while *B. subtilis* was the most sensitive with zone of inhibition of 4.0 mm at 20 mg/ml and 21.0 mm at 100 mg/ml. With FC-4-B22 (ester of glycerol) using paper disc method, *P. aeruginosa* was the most resistant with no zone of inhibition at 20 mg/ml. Meanwhile, *S. aureus* was the most sensitive with zone of inhibition of 3.0 mm at 20 mg/ml. However, *B. subtilis* exhibited a zone of 15.0 mm at 100 mg/ml. Using the agar diffusion method, 7,8,9,-trihydroxy-2,8-dihydroxy naphtha-10-one showed an appreciable effect on the tested pathogens. *Pseudomonas aeruginosa* was the least sensitive with the zone of inhibition of 3.0 mm at 20 mg/ml and 8.0 mm at 100 mg/ml while *S. aureus* was the most sensitive to the extract at 20 mg/ml with the zone of inhibition of 6.0 mm at 100 mg/ml. *Bacillus subtilis* was most sensitive to the extract at 100 mg/ml with the zone of inhibition of 19.0 mm. Testing the efficacy of ester of glycerol using agar diffusion method, *P. aeruginosa* showed the least resistance while *B. subtilis* was the most sensitive.

Conclusion: From this study, it can be concluded that the two novel compounds exhibited differential antibacterial activities towards the test bacteria.

Keywords: Phytoalexin; agar diffusion; paper disc; Theobroma.

1. INTRODUCTION

Cocoa (*Theobroma cacao* L.) has become one of the main cash crops in Nigeria grown mainly in the South West and South Eastern parts of the country. Global annual earnings from export cocoa average US$2.9 billion [1]. The local production of the crop for export is constrained by many diseases and pests [2,3]. The global losses of cocoa due to diseases and pests based on data collected from various cocoa growing countries like Ghana, Nigeria, Sierra Leone, Togo, Trinidad and Tobago and West Cameroon was about 29.4 % of its production [4].

Attack by diseases and pests may result in a direct loss of crop as with *Phytophthora* pod rot (black pod) or the tree may be debilitated as with vascular-streak die back while the trees may even be killed by Ceratocystis wilt [5]. [6] reported that over 60% of fruit set is lost to pests, diseases and cherelle wilt in cocoa plantations. This is the most serious disease of cacao in West Africa especially Nigeria, Ghana and Cameroon caused by *Phytophthora palmivora, P. megakarya, P. capsici* and *P. citrophthora* [7-12]. The disease progresses rapidly from the tip and the whole pod is infected, turns black in about ten days and eventually becomes black and mummified and sometimes covered in a white mass of sporangia [4].

Pods of all ages and sizes are infected. Black pod disease is difficult to control. To withstand the hostile environment of pathogenic microorganisms, plants have evolved several sophisticated physical and biochemical mechanisms against pathogenic infections [13]. Nevertheless, some pathogens seem to be capable of causing a great number of plant diseases. Different plants defend themselves against pathogens in different ways. Each kind of plant probably employs different defense mechanisms against each of the various pathogens that attack it [14]. The reaction of a plant to a pathogen apparently depends on the kind of pathogen but is also influenced by environmental conditions prevalent during infection since they may influence the physiological activity of the plant to a lesser or greater extent [15]. The host plant triggers several biochemical reactions, resulting in the formation of defense chemicals which are aimed at localizing and killing the pathogens [14,16-19]. A very interesting group of such compounds is termed phytoalexin.

These compounds can inhibit the development of pathogenic microorganisms but are only formed or activated when the pathogen comes into contact with the host plant cells. Phytoalexins are non specific toxins and the difference between resistance and susceptible host plants lies in the speed of formation of the compounds or antibiotics. Studies of the antibacterial activities of many phytoalexin have been more restricted in number and scope than investigation into antifungal activities.

[20] screened two phytoalexins namely 3,5-dihydrodiphenyl, l,2 ethane (Dihydropinosylvin) and 3,2,5 trihydroxydiphenyl, 1,2 ethane (Batatasin IV) against *B. cereus*, *S. aureus, P. aeruginosa* and *E.coli* and reported that dihydropinosylvin exhibited stronger antibacterial activity against all the test bacteria than batatasin IV from the tubers of *Dioscorea rotundata*. Furthermore, [21] reported the antibacterial activity of dimetyl batatasin IV and 3,5,4' trihydroxybibenzyl (dihydro resveratrol) stilbene phytoalexins isolated from the bulb of *D.bulbifera* and tuber of *D. dumentorum* and found *E.coli*, *Serratia marcescens* and *S. aureus* to be sensitive but showed no antibacterial activity against *B. subtilis, P, aeruginosa* and *Klebsiella pneumoniae*.

Recently, [22] reported the antibacterial activity of diethyl ether crude extract from cocoa pods *Theobroma cacao L.* infected with *P. palmivora* against *Shigella sonnei, Proteus mirabillis, K. pneumoniae* and *Enterobacter aerogenes* and found the crude extract to be highly active against all the test bacteria.

However, this research was prompted by a lack of published works on the effects of phytoalexin from *Theobroma cacao* L. on bacteria.

2. MATERIALS AND METHODS

2.1 Source of Plant Materials

Fresh healthy mature green cocoa pods were obtained from cocoa plantation of Ado Grammar School, Ado-Ekiti, Nigeria. The pods were immediately transported to the Department of Microbiology Laboratory of the University of Ado Ekiti, Ekiti State and washed with two changes of distilled water. The pods were sundried and later kept in a well ventilated place in the laboratory for 24h.

2.2 Solvents

The solvents used were ethyl acetate (BDH England), n–hexane, methanol and dichloromethane - (hexane: ethylacetate - 9.5: 0.5; methanol: dichloromethane - 0.5:9.5) in the development of thin layer chromatography (T.L.C.) plates and elution of column chromatography (C.C.). Preparative T.L.C. were dipped in acetone and allowed to air dry before use.

2.3 Infection of Cocoa Pods and Extraction of Phytoalexins

Healthy mature cocoa pods were washed with two changes of distilled water and allowed to dry. The cocoa pods were surface sterilized by swabbing with cotton wool moistened with 75% ethanol. A sterile cork borer (6mm diameter) was used to bore hole into the cocoa husk. Mycelial disc containing *Phytophthora palmivora* was inoculated into the hole and the husk was replaced to cover the inoculum. Sterile vaseline cream was applied on the burrowed surface to ensure proper sealing of the area. The infected cocoa pods were placed in a sterile polyethylene bag and sprayed with distilled water daily to humidify it and left to stand at 25°C in the laboratory for 5-7 days [23]. The experiment was carried out in four replicates. For the control experiment, cocoa pods were treated as described above but were not inoculated with mycelia disc but with distilled water. The pods were watered with distilled water. The pods were also put in polyethylene bags and similarly incubated at 25°C in the laboratory for 5 – 7 days.

Eleven kilogrammes of cocoa pod husks were treated for 120 h and later soaked in ethyl acetate. The crude extract was concentrated at 40ºC using a rotator evaporator and the dried crude extract were kept in a refrigerator at 4ºC until ready to use.

2.4 Purification of Crude Extract

Purification of crude extract was carried out by the vacuum liquid chromatography (V.L.C.). The pre adsorbed sample was applied to the top of the packed column. The adsorbent solid (silica) was tightly packed by compressing it with a flat disc [24]. The vacuum pump was switched on and elution carried out successively with the following solvent systems as shown below:

(i) n – hexane 100%
(ii) n – hexane/dichloromethyl (9:1, v/v)
(iii) dichloromethyl 100%
(iv) dichloromethyl/methanol (1:1, v/v)

About 50 ml fractions were collected and examined with T.L.C. in solvent system dichloromethyl: methanol (9.5: 0.5) and spots were detected by examining under ultraviolet (U.V.) light at 254 nm and 365 nm followed by spraying with vanillin/sulphuric acid (2: 1 v/v) reagents and heated in a ventilated oven at

100°C to determine the chromatographic pattern which was used to bulk each of the fractions together.

2.4.1 Purification of Vacuum Liquid Chromatography (V.L.C.) bulked fraction B by preparative T.L.C.

The V.L.C. bulked fraction obtained was dissolved in dichloromethyl and streaked onto 0.5 mm preparative T.L.C. plates and developed in ethyl acetate: n – hexane (0.5: 9.5, v/v). The edges of the T.L.C. developed plates were sprayed with vanillin/conc. sulphuric acid (2:1, v/v) and examined under U.V. light. The R_f values, that corresponded to antifungal zone in the T.L.C. bioassay were marked and scrapped off the glass plates. The compounds were eluted from the powder by washing with dichloromethyl. Dissolved silica gel was removed by filtering through non-absorbent cotton wool. The filtrate was finally reduced to dryness *in vacuo* at 40°C.

The residue was further spotted on analytical T.L.C. plates and developed in ethyl acetate: n – hexane (0.5: 9.5, v/v). The developed plates were further examined under U.V. light (λmax 265 and 365) and sprayed with vanillin/Conc. Sulfuric acid (2: 1, v/v) to check the purity of the compounds.

This procedure was repeated several times until the pure compounds were obtained. The compounds were bioassayed on T.L.C. plates to check if they still retained their activity. The compounds were also spotted and sprayed with the following spraying reagents: Fast blue B salt (FBB), p – nitroaniline (Diazotized) buffered (D.P.N.) and Dragendorff reagent [24] to check their chemical nature.

2.4.2 Spectroscopic techniques

Nuclear Magnetic Resonance (NMR) spectra were obtained using 200 MHz Varian, 300 and 600 MHz Bruker spectrometers. Spectra editing in ^{13}C NMR was done using distortion less enhancement by polarization transfer (DEPT) technique. For some compounds, two dimensional (2D) NMR experiments were carried out.

2.5 Determination of Antibacterial Activity of the Purified Compounds

2.5.1 Source of microorganisms

The bacterial cultures used were *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus*, and *Micrococcus luteus*. These organisms were obtained from the stock cultures maintained on nutrient agar and stored at 4ºC in the Department of Microbiology, Obafemi Awolowo University, Ile Ife, Nigeria. Monthly sub-culture was carried out on nutrient agar.

2.5.2 Standardization of bacterial inocula

To standardize the density of inoculum for the susceptibility test, a $BaSO_4$ turbidity standard equivalent to 0.5 McFarland standard or its optical equivalent was used [25]. A $BaSO_4$ 0.5 McFarland was prepared as follows: exactly 0.5 ml aliquot of 0.048 mol/L $BaCl_2$ (1.175% w/v $BaCl_2.2H_2O$) was added to 99.5 ml of 0.108 mol/L H_2SO_4 (1% v/v) with constant stirring to maintain a suspension. The correct density of turbidity standard was verified by using a spectrophotometer (PYE Unicam Sp, U.K.) with a 1 cm light path and matched cuvette to determine the absorbance at 625 nm was 0.08. The $BaSO_4$ solution was transferred in 4-6 ml aliquot into screw cap tubes of the same size as those used in growing or diluting the bacterial inoculum. The tubes were tightly sealed and stored in the dark at room temperature. The barium sulphate turbidity standard was vigourously agitated for a uniform turbid appearance before use. Latex particle suspension was mixed by inverting gently, and not on a vortex mixer.

2.5.3 Paper disc method

The methods of [26] were used. A sterile pair of forceps was used to load each disc with a prescribed amount of the compound in dichloromethyl (20, 40, 60, 80 and 100 mg/ml). The discs, loaded with the compounds were allowed to dry before placing them on dried nutrient agar plates seeded with each bacterium. In each plate, there was a control disc placed at the centre of the Petri dish which was dipped in dichloromethane only. The plates were incubated at 37ºC and the diameter of zones of inhibition was measured after 24h.

2.5.4 Agar diffusion method

The method of [27] was used. Different concentrations of each compound 20, 40, 60, 80 and 100 mg/ml were introduced into each of the five wells and the sixth well was filled with dichloromethane which served as control. This method was carried out for each of the test bacteria. The plates were then incubated at 37ºC

for 24h after which the zones of inhibition were measured.

3. RESULTS AND DISCUSSION

3.1 Time Course Study of Phytoalexin Production

There was no trace of phytoalexins production after 48h of induction, at 72 h post infection, one spot of reddish/pink colour was observed and five spots of reddish/pink colour were noticed after 120 h. Furthermore, 144 h post infection/induction, only two spots were detected while the extracts of 168 h post inoculation showed no spot at all (Table 1). However all the spots (reddish/pink colour) showed antifungal activities.

Table 1. Colour reaction of phytoalexin to vanillin/conc. H_2SO_4 at various periods of incubation

Period of incubation (h)	Colour reaction
48	Nil
72	Red
96	Nil
120	Red, Pink
144	Red, Pink
168	Nil

3.2 Spectral Data of Compound FC-3-B21(I)

Yield:
UV spectum: ND
M.S. ND
^{1}Hnmr-(CDCl3, 100MHz) 1.55(m, 4H,J $2CH_2$) 2.93(d, 2H, J = $7.3H_z$, CH_2) 4.75 (1H, dd, J = 6.8, $7.3H_z$ CH0H)
6.75(d, 1H, J = $7.4H_z$, = CH) 6.90 (1H,d, J = $8.6H_z$ =CH)
7.42(1H,t, J = 8.1, $7.6H_z$ = CH)
^{13}Cnmr(CDd_3): δ 21.02 (CH_2), 29.96(CH_2) 34.84(CH_2); 76.37 (CH0H), 116.48 (CH)118.15(CH) 136.41 (CH).

The results of the antibacterial activity of 7,8,9,-trihydroxy-2,8-dihydroxy naphtha-10-one (I) using paper disc method are shown in Table 2. The test bacteria exhibited differential sensitivity to compound (I) at various concentrations. All the test bacteria were sensitive to the extract even at the lowest concentration of 20 mg/ml. *Pseudomonas aeruginosa* showed the least sensitivity of 2.0 mm zone of inhibition and 12 mm at 100 mg/ml while *B. subtilis* was the most sensitive with zone of inhibition of 4.0 mm at 20 mg/ml and 21.0 mm at 100 mg/ml.

The results of the antibacterial activity of ester of glycerol (II) using paper disc method are shown in Table 3. All the test bacteria were sensitive to the extract at the various concentrations. *Pseudomonas aeruginosa* was the least sensitive with no zone of inhibition at 20 mg/ml but with 4.0 mm at 40 mg/ml and 8.0 mm at 100 mg/ml while *S. aureus* was the most sensitive to the extract with zone of inhibition of 3.0 mm at 20 mg/ml while *B. subtilis* had a zone of 15.0 mm at 100 mg/ml.

The results of the antibacterial activity of 7,8,9,-trihydroxy-2,8-dihydroxy naphtha-10-one (I) using agar diffusion method are shown in Table 4 while the results of the ester glycerol are shown in Table 5. The test bacteria were all sensitive to the extract at various concentrations with *P. aeruginosa* being the least sensitive with the zone of inhibition of 3.0 mm at 20 mg/ml and 8.0 mm at 100 mg/ml. *S. aureus* was the most sensitive to the extract at 20 mg/ml with the zone of inhibition of 6.0 mm while *B. subtilis* was most sensitive to the extract at 100 mg/ml with the zone of inhibition of 19.0 mm.

Table 2. Antibacterial activity of 7,8,9,-trihydroxy-2,8-dihydroxy naphtha-10-one using paper disc method (zone of inhibition in mm)

Test organisms	Conc. of purified compounds (mg/ml)					
	20	40	60	80	100	Cc
Zone of Inhibition (mm)						
Bacillus subtilis	4.0	10.0	14.0	17.0	21.0	0.992
Escherichia coli	3.0	7.0	12.0	16.0	19.0	0.996
Micrococcus luteus	4.0	8.0	14.0	16.0	16.0	0.943
Pseudomonas aeruginosa	2.0	5.0	9.0	11.0	12.0	0.977
Staphylococcus aureus	4.0	9.0	12.0	14.0	16.0	0.977

Cc = Correlation coefficient

Table 3. Antibacterial activity of ester of glycerol using paper disc method

Test organisms	Conc. of purified compounds (mg/ml)					
	20	40	60	80	100	Cc
Zone of Inhibition (mm)						
Bacillus subtilis	2.0	8.0	10.0	12.0	15.0	0.97
Escherichia coli	2.0	6.0	8.0	11.0	13.0	0.99
Micrococcus luteus	2.0	6.0	7.0	10.0	11.0	0.976
Pseudomonas aeruginosa	0.0	4.0	4.0	6.0	8.0	0.959
Staphylococcus aureus	3.0	7.0	8.0	7.0	9.0	0.083

Cc = Correlation coefficient

Table 4. Antibacterial activity of 7,8,9,-trihydroxy-2,8-dihydroxy naphtha-10-one using agar diffusion method

Test organisms	Conc. of purified compounds (mg/ml)					
	20	40	60	80	100	Cc
Zone of Inhibition (mm)						
Bacillus subtilis	5.0	10.0	13.0	16.0	19.0	0.993
Escherichia coli	4.0	8.0	11.0	15.0	18.0	0.994
Micrococcus luteus	4.0	9.0	11.0	13.0	15.0	0.974
Pseudomonas aeruginosa	3.0	5.0	6.0	7.0	8.0	0.986
Staphylococcus aureus	6.0	7.0	9.0	10.0	13.0	0.770

Cc= Correlation coefficient

Table 5. Antibacterial activity of ester of glycerol using agar diffusion method

Test organisms	Concentration of purified compounds (mg/ml)					
	20	40	60	80	100	Cc
Bacillus subtillis	3.0	8.0	9.0	11.0	13.0	0.965
Escherichia coli	2.0	6.0	8.0	9.0	12.0	0.979
Micrococcus luteus	3.0	7.0	8.0	9.0	11.0	0.959
Pseudomonas aeruginosa	1.0	2.0	4.0	7.0	8.0	0.985
Staphylococcus aureus	2.0	4.0	6.0	8.0	11.0	0.996

3.3 Characterization of Compound FC–3–B21

Compound FC – 3 – B21 was basically characterized using the ^{1}Hnmr and ^{13}Cnmr as well as the 2D cosy data. The ^{1}Hnmr showed an aromatic ABX system of 1H each indicating substitute in1, 2, 3 positions. The signal at δ 11.0 showed that the compound is a phenol as it represents phenolic OH in an H – bounding position. This was confirmed in the ^{13}Cnmr with δ 136.4 indicating an aromatic phenol which was assigned to position 5.

Thus, the none aromatic position of the compound was joined at position 1and 2. Further analysis of the ^{1}Hnmr spectrum showed that the CHOH signal at δ 4.75 is coupled to at least 2CCH$_2$ groups as the splitting pattern of a dddd indicated, hence the deduction of the X – CH$_2$-CHOH–CH$_2$-character. The ^{13}Cnmr confirmed the deduction above with corresponding signals. However it also had an additional signal at δ 21.02 deduced as an extra – CH$_2$ group since the ^{1}Hnmr signal at 1.55 is a multiplex instead of a clear doublet. Thus, compound FC-3-B21 was characterized as 7,8,9,-trihydroxy-2,8-dihydroxy naphtha-10-one The corresponding structure is shown in Fig 1.

3.4 Spectral and Structural Characterization of Compound FC – 4 – B22

^{1}Hnmr (CDCL$_3$) δ 0.9 (9H, m, 3 x XH$_3$); 1.15(17H, m, CH$_2$) 1.6(2H, m,) 2.0, (3H, m) 2.4 (2H, m) 4.2 (2H, m), 5.4 (m, CH). But it apparently contains, double unsaturation with hydroxyl groups as shown by signals between 128 and 132 and has terminal CH$_3$ groups. The fully acid component has a minimum of 16 carbon units. Thus, compound FC-4-B22 was characterized as ester glycerol. The corresponding structure is shown in Fig. 2.

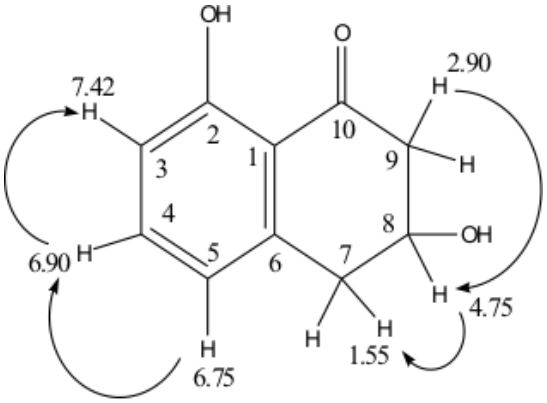

Fig. 1. Structural formula of 7,8,9-trihydroxy-2,8-dihydroxynaph-10-one

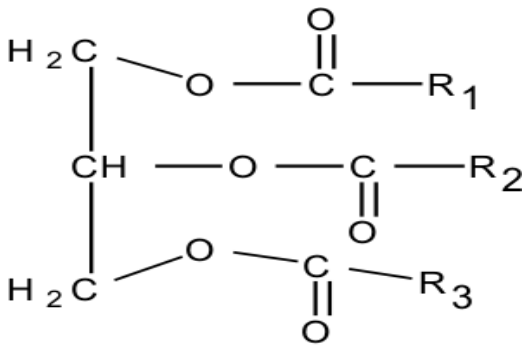

Fig. 2. The Structural formula of Ester of Glycerol

The differential sensitivity to 7,8,9,-trihydroxy-2,8-dihydroxy naphtha-10-one. (compound I) at various concentrations by the test bacteria is similar to the work of [22] who reported an increase in the zone of inhibition of the test bacteria (*Enterobacter aerogenes, Klebsiella pneumoniae, Proteus mirabilis* and *Shigella dysenteriae*) when the concentration of the crude extract from cocoa pods infected with *P. palmivora* was increased. In addition, [28] reported a progressive increase in the zone of inhibition with increased concentration of ethanolic leaf extract of whole plant *Indonessiiella echioides* on the test bacteria. This result is also similar to the findings of [29] who reported the antibacterial activities of momilatones A (M_A) and B (M_B) which are phytoalexins derived from the rice plant (*Oryza sativa*) against *P. ovalis, B. cereus, B. pumilus* and *E. coli.* However, momilatone B (M_B) exerted significant stronger antibacterial activities than momilatone A (M_A) against the test bacteria.

Also examining the antibacterial activity of an ester of glycerol (compound II) using paper disc method, results showed that all the test bacteria were sensitive to the extract at various concentrations. This is similar to the findings of [30] who reported that *Xanthomonas campestris* was sensitive to all the concentrations of leuttucenin A, a phytoalexin induced by abiotic and biotic factors from *Lactuca sativa*. Also [31] reported an increase in the zone of inhibition on *Erwina* spp*., X. campestris, Clavibacter michiganese and P. syringe* when administered with increased concentration of leaf extract of *Artenisia nilagirica*.

Using agar diffusion method, all the test bacteria were sensitive to the extract at various concentrations of 7,8,9,-trihydroxy-2,8-dihydroxy naphtha-10-one. With *P. aeruginosa* the compound was less active (3.0 mm at 20 mg/ml and 8.0 mm at 100 mg/ml). *Staphylococcus aureus* was more sensitive to the extract at 20 mg/ml while *B. subtilis* was the most sensitive to the extract especially at 100 mg/ml with the zone of inhibition of 19.0 mm. The result of this work is in conformity with the findings of [32] who reported the growth inhibition of *S. aureus, Enterococcus faecalis* and *P. aeruginosa* by resveratrol at 171-342 μg/ml. It also in agreement with the work of [33] who reported that the crude methanol extract and the n-butanol fraction of *Ingofera pulchra* showed strong antimicrobial activity against *S. aureus, P. aeruginosa* and *B. subtilis*.

Antibacterial activity of an ester of glycerol may be due to the ability of *P. aeruginosa* to metabolise the extract. *Pseudomonas aeruginosa* had earlier been reported by [34] to degrade most plant products and render them effective in terms of antibacterial activity. Similarly, [35] also reported the susceptibility of *B. cereus* to the leaf extracts of *A. douglasiana.*

4. CONCLUSION

This study showed that the two novel compounds exhibited differential antibacterial activities towards the test bacteria and could be a source of antibiotics.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gray A. The world cocoa market outlook. Ghana Conference paper, LMC International Press; 2000. Available:http://www.treecrops.org/crops/cocoaoutlook
2. Okaisabor EK. *Phytophthora* pod rot infection from soil in "Phytophthora diseases of cocoa" Edited by P.H. Gregory. Longman Group Ltd, London. 1974;161–168.
3. Wood GAR, Lass RA. *Cocoa*. 4th Edition, Oxford University Press, London. 1985;83.
4. Opeke LK. Tropical tree crops. Woye and Son Ltd, Ilorin, Nigeria. 1992;109.
5. Phillips TI. An Agricultural Note Book. Longman Nigerian Ltd, Ibadan, Nigeria; 1984.
6. Olaiya AO, Fagbayide JA. Pod yield and losses in cocoa production in South Western Nigeria. In Proceedings of the 14th International Cocoa Research Conference. Accra, Ghana. 2003;1285-1289.
7. Nyasse S, Cilas C, Herail C, Blaha G. Leaf inoculation as an early screening test for cocoa (*Theobroma cacao* L.) resistance to *Phytophthora* black pod disease. Crop Protection. 1995;14:657–663.
8. Arowolo JA, Fagbohun ED. Occurrence and distribution of *Phytophthora* canker on cocoa in Ekiti State, Nigeria. African Journal of Science. 2001;1(1):100–106.
9. Omokolo ND, Nankeu DJ, Niemenak N, Djocgoue PF. Analysis of aminoacid and carbohydrate in the Cortex of nine clones of *Theobroma cacao* L. in relation to their susceptibility to *Phytophthora megakarya* Bra. and Grif. Crop Protection. 2002;21: 395–402.
10. Omokolo ND, Nankeu DJ, Niemenak N, Boudjeko T. Variation of β– 1,3 – glucanase, chitinase and polyphenol oxidase activities in cacao pods upon *Phytophthora megakarya* inoculation. African Crop Science Journal. 2003;11:97–106.
11. Boudjeko T, Djocgoue PF, Nankeu JD, Mbouobda HD, Omokolo DN, Hadrami IEL. Luteolin derivatives and heritability of resistance to *Phytophthora megakarya* in *Theobroma cacao* L. Australasian Plant Pathology. 2007;36:56-61.
12. Fagbohun DE, Falegan CR. Arowolo JA. *Phytophthora* black pod disease in Ekiti State of Nigeria- Species differentiation and pathogenic variation. Bioscience, Biotechnology Research Asia. 2008;5(1): 157-160.
13. Soylu S, Bennet MH, Mansfield JW. Induction of phytoalexin accumulation in broad bean (*Vicia faba L*) cotyledons following treatment with biotic and abiotic elicitors. Turk. J. Agric. 2002;26:343-348.
14. Agrios GN. Plant pathology. Academic Press, San Diego, C.A., USA; 2005.
15. Gonzalez-Lamothe R, Michell G, Gattuso M, Diarra MS, Malouin F, Bouarab K. Plant antimicrobial agents and their effects on plant and human pathogens. Int. J. Mol. Sci. 2009;10:3400–3419.
16. Nicholson RL, Hammerschmidt R. Phenolic compounds and their role in disease resistance. Ann. Rev. Phytopathol. 1992;30:369–389.
17. Bailey JA, Mansfield JW. *Phytoalexins*. Blackie, London and Glasgow. 2001;100-105.
18. Hammer – Schmidt R. Phytoalexins: what have we learned after 60 years? Ann. Rev. Phytopathol. 1999;37:285–306.
19. De Ascenasao A, Dubery IA. Soluble and wall – bound phenolics and phenolic polymers in *Musa accuminata* roots exposed to elicitors from *Fusarium oxysporum* F.sp. *cubense*. Phytochem. 2003;63:679–683.
20. Fagbohun DE, Ogundana SK, Adesanya SA, Roberts MF. Dihydrostilbene phytoalexin from *Dioscorea rotundata.* Phytochem. 1987;26(12):3187–3189.
21. Adesanya SA, Ogundana SK. Roberts MF. Dihydrostilbene phytoalexin from *Dioscorea bulbifera* and *D. dumentorum*. Phytochem. 1989;28(3):773-774.
22. Duyilemi OP, Fagbohun DE. Antibacterial activity of crude extract from infected cocoa pods. J. Appl. Trop. Agric. 2007; 12(1,2):28-31.
23. Saskia CM, Wees V, Chang, H, Zhu T, Glazebrook J. Characterization of early response of Arabidopsis to *Alternaria brasicicola* infection using expression profiling. Plant Physiol. 2003;132;606-617.
24. Harbone JB. Phytochemical methods. Chapman and Hall Ltd., London; 1982.
25. Bauer AW, Kirby WW, Shorries JC, Turicks M. Antibiotics susceptibility testing by a standard single disc method. Amer. J. Clinc. Path. 1996;45:493-496.
26. Fagbohun ED, Falegan CR, Arowolo JA. Antimicrobial activities of the extracts of the peels of *Dioscorea dumentorum* Pax.

Bioscience Research Communications. 2004;16(1):69–75.
27. Isu NR. Antibacterial effect of *Aframomum melaguata, Xylopia aethiopicum* and *Ocimum viride.* Nig. J. Nat. Prod. and Med. 2005;9:22–25.
28. Ratthman A. Antimicrobials in Laboratory Medicine. Churchill Living-Stone, Press. Ltd, New-Delhi. 2000;194-203.
29. Fukuta M, Xuan TD, Deba F, Tawata S, Khanh TD, Chung IM. Comparative efficacies in vitro of antibacterial, fungicidal, antioxidant and herbicidal activities of momilatones A and B. Journal of Plant Interractions. 2007;2(4):245-251.
30. Yean CH, Atong M, Chong PK. Lettucenin A and its role against *Xanthomonas campestris.* J. of Agric. Sci. 2009;1(2):87–93.
31. Cha JD, Jung EK, Kil, Lee KY. Chemical composition and antibacterial activity of essential oil from *Artemisia feddei*. J. Microbial Biotechnology. 2007;17:2061-2065.
32. Chan MM. Antimicrobial effect of resveratrol on dermatophytes and bacterial pathogens of the skin. Biochem. Pharmacol. 2002;63:99–104.
33. Musa AM, Ilyas M, Haruna AK, Iliya I, Akpulu IN. Phytochemical and antimicrobial activity studies on *Indigofera pulchra*. Inter. J. Pure and Appl. Sci. 2007; 1(3):25–30.
34. Manou I, Bouillard L, Devleeschouwer MJ, Barel AO. Evaluation of the preservative properties of *Thymus vulgaris* essential oil in topically applied formulations under a challenge test. J. Applied Microbiol. 1998; 84:368-376.
35. Setzer WN, Vogler B, Schmidt JM, Leahy JG, Rives R. Antimicrobial activity of *Artemisia douglasiana* leaf essential oil. Fitoterapia. 2004;75:192-200.

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