

4 **Antibacterial activity of bioflavonoid from fruit pulps of *Acacia nilotica* Willd**

5 **Abstract**

6 The emergence of multi-drug resistance in bacteria has led to call for research and development
7 of new leads as antibiotics from medicinal plants. *Acacia nilotica* (Linn) is a plant of
8 multipurpose medicinal uses, three bioactive flavonoids (methyl gallate, gallic acid and catechin)
9 were isolated from its fruit pulps through a bioassay guided fractionation technique and
10 characterized based on High Performance Liquid Chromatography, Liquid Chromatography-
11 Mass Spectra and Nuclear Magnetic Resonance spectra. Antibacterial activity of these
12 compounds was determined by microplate tetrazolium dye assay of broth microdilution
13 technique against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and
14 clinical isolates of *Salmonella typhi*, *Klebsiella pneumonia*, *Candida albicans* and *Bacillus*
15 *subtilis*. Catechin, methyl gallate and gallic acid at 19.5, 39 and 39 µg/ml respectively caused a
16 significant bio-reduction in cells of test organisms. Time kill kinetic study of the extract shows
17 that there was percentage of growth reduction in test organisms at 2, 4, 6, 8 and 12 hrs of contact.
18 The extent and rate of killing of the organism by the extract at 2 x MIC followed the same trend
19 as rate of killing was time dependent. Antibacterial effects of these compounds are within the
20 breakpoint of control drug chloramphenicol and could serve as leads in new drug development.

21 **Keywords:** *Acacia nilotica*, antibacterial, catechin, methyl gallate and gallic acid, bioactive
22 compound, high through put techniques.

23 1.0 Introduction

24 *Acacia nilotica* Willd. Ex Delile is commonly known as Gum Arabic tree, Egyptian
25 mimosa, Egyptian thorn, red thorn, Babool, babul in Indian [1]. It is recognized by the following
26 names: Acacia, Acacia Arabica, Babhul – Hindi and Nepalese, Babool Baum – German etc []. It
27 has naturalized in several countries where it has been introduced as a medicinal, forage and fuel
28 wood plant [2; 1]. It is proverbial, medium sized tree and is broadly scattered in tropical and
29 subtropical countries. It has an inspiring range of medicinal uses with potential antioxidant
30 activity. It contains a profile of a variety of bioactive components such as gallic acid, (+)-
31 catechin and methyl gallate. It also contains L-arabinose, catechol, galactan, galactoaraban,
32 galactose, N-acetyl djenkolic acid, sulphoxides and pentosan. The seeds contain crude protein
33 (18.6%), ether extract (4.4%), fiber (10.1%), nitrogen-free extract (61.2%), ash (5.7%) and silica
34 (0.44%); phosphorus (0.29%) and calcium (0.90%) of dry matter (17%) [3]. Phytochemical
35 analysis of stem bark of *A. nilotica* showed the presence of carbohydrates, saponins, tannins, and
36 cardiac glycosides [4].

37 As a multipurpose medicinal plants, leaves, flowers, seeds, roots, fruits and gum act as
38 anti-tuberculosis [5], anti-hypertensive and anti-spasmodic, , antibacterial and antifungal [6],
39 antiplasmodial [7], and antioxidant [8, 9, 10]. Toxicologically, *A. nilotica*, at 2 and 8% levels,
40 has a low toxicity potential [11]. In a survey of potentially allergenic plants in Pondicherry, it
41 was reported likely to cause pollen allergy [12]. *A. nilotica* has a wealth of medicinal uses for
42 stomach upset and pain, the bark is chewed to protect against scurvy, an infusion is taken as
43 therapy for dysentery and diarrhea [13]. In Nigeria, it is one of the customary drugs for treating
44 diarrhea and it has been authenticated to have an antidiarrheal property [3] and antimicrobial
45 activity against neuro-pathogenic *E. coli* K1, MRSA and *Klebsiella pneumonia* [14].

46 This study describes the inhibitory effect of bioactive molecules of *A. nilotica* as leads for drug
47 development against pathogenic organisms using tetrazolium salts reduction as indicators of cell
48 inhibition.

49 **2.0 Methodology**

50 **2.1 Chemicals/Reagents/equipment:** Tetrazolium dye, Nutrient broth No 1, Muller Hinton
51 Agar from Fluka Analytical, Sigma-Aldrich Co., USA; Nutrient Agar from HIMedia
52 Laboratories Pvt Ltd., India; chloramphenicol disk (HIMedia Laboratories Pvt. Ltd, India),
53 Uv/vis spectrophotometer (Jenway 6405, Britain), nuclear magnetic resonance (300 MHz,
54 Varian machine), HPLC and LCMS.

55 **2.2 Extract preparation and isolation:** The pulp of *A. nilotica* fruit was collected from Suleja,
56 Niger state, Nigeria, identified at Herbarium Unit of National Institute for Pharmaceutical
57 Research and Development, Abuja, Nigeria and herbarium specimen was deposited. This part
58 was air dried to constant weight at room temperature and pulverized. Extraction of both polar
59 and non-polar components of the plants using 70% aqueous methanol was carried out. One
60 hundred grams of dried plant part was soaked in 70% aqueous methanol (1000 ml) for 24 hrs.
61 The extract was filtered, concentrated on rotary evaporator at 40°C and freeze dried. A portion of
62 the crude extract was used to determine the preliminary antibacterial effect of the plant, while the
63 other portion was subjected to bioassay guided isolation of the bioactive molecules (Figure 1).

64
65 Fractionation of the crude extract of *A. nilotica* (seeds) was carried out according to [15].

66 Activated silica gel (50 g) was packed by a wet method into a column. The extract (2.6g) which
67 was absorbed on silica gel (60 – 120 mesh) and dried was loaded on the column. Gradient
68 elution was performed with 100 ml of each mobile phase mixture in a series. The elution was

69 performed in a flash chromatography. The mobile phase consisted of hexane, ethyl acetate,
70 methanol and water, starting from 100% hexane and 10% increment in the next polar component.
71 The final elution was performed with 70% methanol in water until the column appeared
72 exhausted with a sign of colourless silica gel. The eluates were monitored by thin layer
73 chromatography (TLC) using normal phase pre-coated silica gel K5 TLC plates. The TLC
74 mobile phase consists of a mixture of ethyl acetate: hexane (7:3). The eluates were combined
75 based on the similarity of TLC fingerprint to give six fractions labelled as FR1- FR6. The pooled
76 fractions (0.8 g) were absorbed on Merck – Kiesegel and introduced into column packed with the
77 same absorbent. It was eluted with petroleum ether and an increasing gradient of ethyl acetate.
78 A total of 6 fractions of 100 ml each were collected. Fractions with same similarity (2-4) were
79 pooled together into 3 portions viz; P1, P2 and P3 based on their TLC profile (Figure 2). On
80 staining with ferric chloride solution showed blue black colouration which is an indication of
81 phenolic compounds. These fractions were purified further by HPLC technique by separating on
82 a reverse phase column, eluting with a gradient of acetonitrile 5% - 98% in water for a period of
83 40 minutes. Some 100 mg of extract was dissolved in 1 ml of methanol, and water was added
84 gradually to the point of precipitation before injecting on the HPLC reversed phase (C-18)
85 column. Fractions were collected at intervals of one minute. The structural elucidation of the
86 compounds was done using Nuclear Magnetic Resonance (300 MHZ, Varian machine) and LC-
87 MS techniques. [2008]. The pure fractions from TLC analysis were dissolved in deuterated
88 methanol (CD₃OD) and transferred to NMR tube for measurement of proton (¹H) and ¹³C
89 (carbon 13). Masses were acquired using liquid chromatography- Mass Spec (LC-MS).

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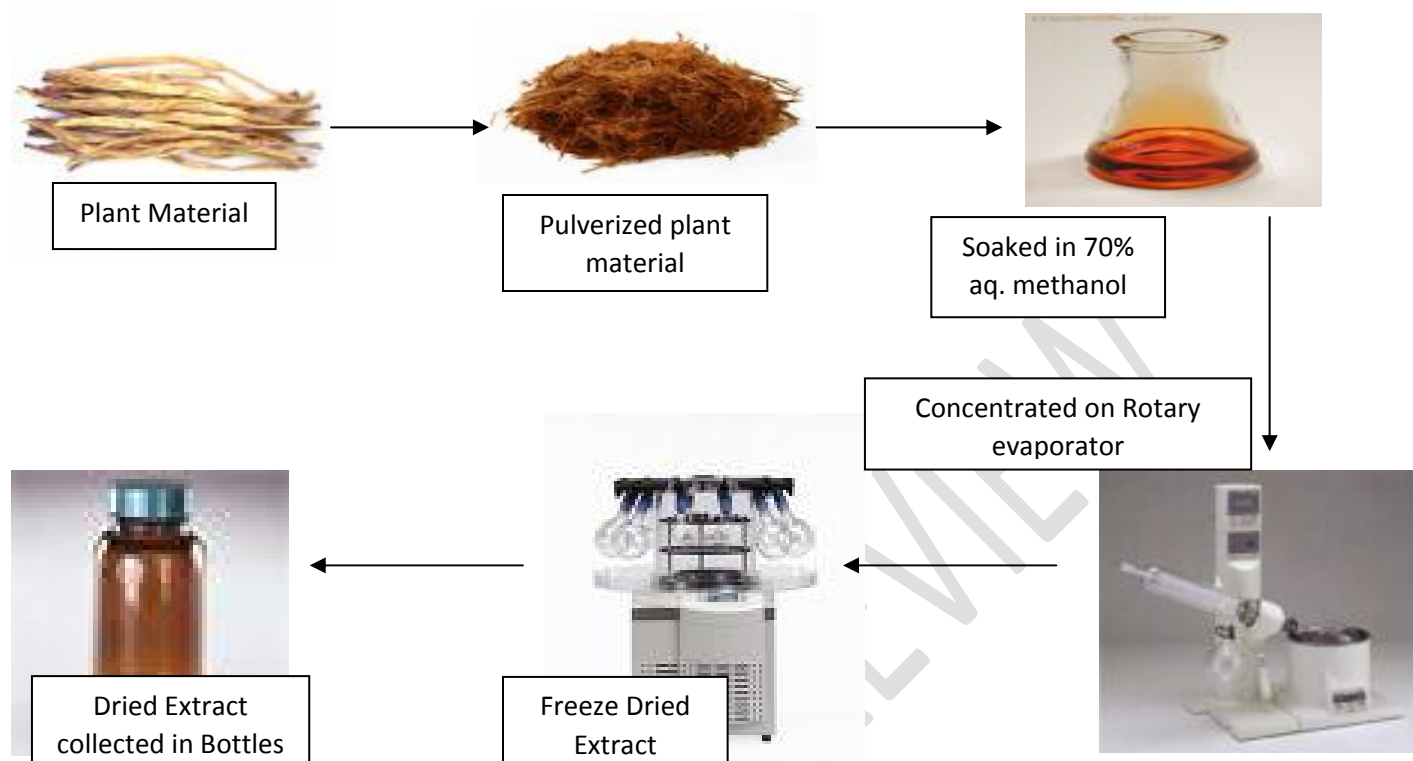


Figure.1. Extraction procedure of fruit pulps of *A. nilotica*

2.3 Phytochemical analysis: Phytochemical analysis of fruit pulp of *A. nilotica* was carried out according to Trease and Evans [16]. Metabolites tested for include alkaloid, saponins, tannins, anthraquinone, cardiac glycosides and flavonoids.

2.4 Antibacterial activity of the crude extract

2.4.1 Extract solution preparation: The extract was dissolved in dimethylsulphoxide (DMSO) and further diluted to 40 mg/ml concentration. The extract was sterile-filtered with 0.22 μ m syringe filter (Fisher brand). From this, a 2 mg/ml concentration of extract in agar was prepared by dispensing 1ml of the extract solution (40 mg/ml) into 19 ml of molten Mueller Hinton Agar, maintained in water bath at 45°C. The molten agar/extract was poured into sterile Petri dish, allowed to gel and dried to remove moisture. Overnight broth cultures of the test organisms viz;

105 *S. aureus* (ATCC 28923), *P. aeruginosa* (ATCC 27853), *E. coli* (ATCC 25922) and clinical
106 isolates of *S. typhi*, *K. pneumonia*, *C. albicans* and *B. subtilis* were standardized by diluting to
107 10^7 cfu/ml using a uv/vis-spectrophotometer as described by [17]. Two to three colonies of 20 hr
108 growth on Mueller Hinton agar of the organisms to be studied were suspended on 50 ml pre-
109 warmed (37°C) Mueller Hinton broth. The suspension was incubated overnight at 37° C, diluted
110 1/2500 in the same pre-warmed medium and incubated in water bath with agitation (50 rpm).
111 The optical density of the culture was monitored at 450 nm until absorbance of 0.1 was reached
112 (equivalent $2.5-3.0 \times 10^7$ cfu/ml for *E. coli* and *P. aeruginosa*, $1.8-2.0 \times 10^7$ for *S. aureus* and *B.*
113 *subtilis*). The agar containing extract was inoculated by dropping 50 µl of standardized organism
114 on the surface of the agar, allowed to stand for 2 hrs. The plate was incubated for 24 hrs at 37°C.
115 The experiment was done in duplicate. Control plates containing 30 µg/ml concentration of
116 chloramphenicol was used as drug control to confirm the susceptibility of the organisms. Agar
117 containing solution of the extract served as extract sterility control. Post incubation, the presence
118 or absence of growth of colonies of test organisms on agar plates indicated positive or negative
119 results of the crude extract.

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121 **2.4.2 Minimum inhibitory concentration of molecules from *Acacia nilotica* (P1, P2 and P3)**

122 The minimum inhibitory concentration of molecules of *A. nilotica* was determined by broth
123 microdilution method according to British Society of Antimicrobial Susceptibility [18]
124 recommendation with a slight modification using tetrazolium colorimetric assay as
125 growth/inhibition indicator [19; 20]. Ten milligrams of each molecule was dissolved in Muller
126 Hinton broth. 100 µl of each of the solutions was transferred to the first column of micro well
127 plate, from where 50 µl was transferred to 2nd well already containing 50 µl Muller Hinton broth,

128 mixed thoroughly and the procedure was repeated through to well 11 where 50 µl was
129 discarded. All the wells were inoculated with 50 µl of overnight diluted cultures of each of *S.*
130 *aureus*, *Pseudomonas aeruginosa*, *E. coli*, *S. typhi*, *K. pneumonia*, *C. albicans* and *B. subtilis*
131 prepared as described above. The plates were incubated for 24 hrs at 37°C. Post incubation the
132 plates were stained with tetrazolium dye; 3-(4, 5-dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium
133 (MTT). MICs were defined as lowest concentration of antimicrobial agents that red formazan of
134 MTT was not observed.

135 **2.4.3 Time kill Kinetic antibacterial study of fruit extract of *Acacia nilotica***

136 One hundred milligram per ml concentration of the crude extract was prepared in sterile water
137 and diluted to 2 mg/ml concentration (being 2 X mic of the crude extract) filtered by centrifuging
138 for 10 mins at 4500 rpm to remove woody sediments. The filtrate was inoculated with 10⁵ cfu/ml
139 of *S. aureus* (ATCC 28923) and *E. coli* (ATCC 25922) and incubated at 37°C, percentage
140 growth decrease was measured at 2 hrs interval. Exactly 0.5 ml of each suspension was
141 withdrawn at the appropriate time intervals and transferred to 4.5 mL nutrient broth recovery
142 medium containing 3% Tween 80 to neutralize the carry-over effects of the antimicrobial
143 compounds from the test suspensions. The suspension was shaken properly then serially diluted
144 in sterile physiological saline. Exactly 0.5 mL of the final dilution of the test organism was
145 transferred into pre-sterile Nutrient agar at 45°C and plated out. The plates were allowed to set
146 and incubated upside down at 37°C for 72 h. Optical density of growing culture in extract
147 solution was measured using uv-spectrophotometer (Jenway 6405 uv/vis, UK) at 520 nm to
148 determine decrease in cells growth. Control experiment which was set up without the inclusion
149 of antimicrobial agent (i.e. excluding the crude extract). Viable counts were made in triplicates
150 for each sample. Depression in the viable counts indicated killing by the antimicrobial agent.

151 Statistical analyses were performed with SPSS version 16.0 (SPSS Inc., Chicago, IL, USA)
152 software. The level of significance for all statistical tests was set at $p < 0.05$.

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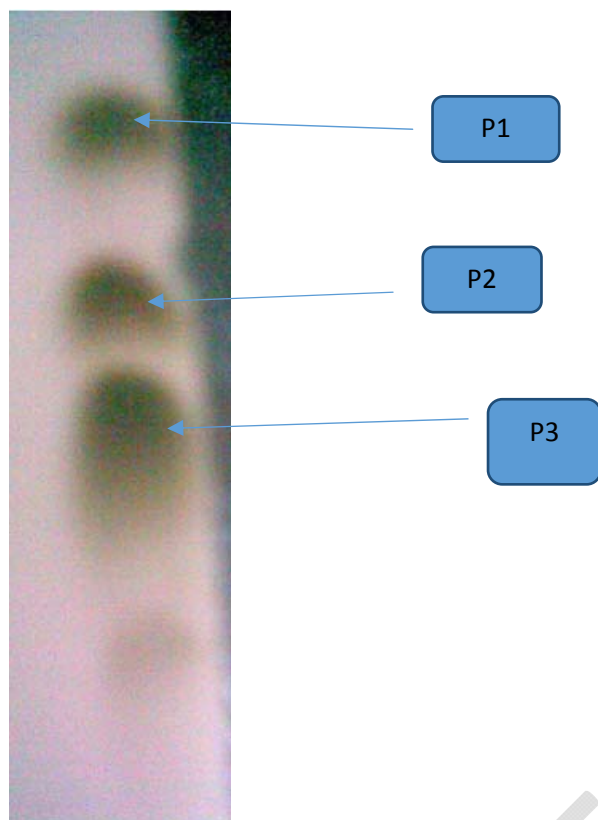
155 **3.0 Results and Discussion**

156 Phytochemical analysis of fruit pulp of *A. nilotica* showed that the plant contains
157 alkaloid, saponins, tannins (phlobatannins), anthraquinone and flavonoids. The presence of these
158 phytochemicals could be responsible for the observed activity. For instance, tannins
159 therapeutically have antiseptic properties and their precipitating quality is used in detecting
160 gelatin, proteins and alkaloids [21]. Flavonoids and phenolic compounds are plants metabolites
161 with at least one hydroxyl group [22] and are often found effective *in vitro* as antimicrobial
162 substance against a wide array of microorganisms [23]. The presence of these phytochemicals
163 could be responsible for the antibacterial activity recorded in this study.

164 Bioassay guided fractionation of the crude extract of fruit pulp of *A. nilotica* yielded 3
165 distinct spots on TLC (Fig. 2). The compounds were analyzed on Liquid Chromatography- Mass
166 Spectrometer (LCMS) for purity and mass. Three compounds were isolated from the fractions by
167 the preparative. The pure compounds were fractions 5, 9 and 12. Fractions in between were
168 mixtures of these compounds as indicated by NMR spectra.

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170 The ¹HMR of fractions 5 revealed it to be methyl gallate, 9 to be gallic acid and 12 to be
171 catechin through a library search [24, 15].



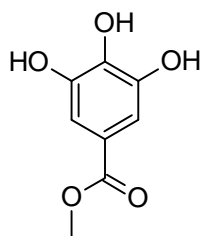
172
 173 **Figure 2. Thin Layer Chromatography plate of active fraction of *A. nilotica* (showing 3**
 174 **distinct spots P1, P2 and P3).**

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 176 **3.1 Characterization of Isolated Compounds:**

177 The first compound P1 (fraction 5) was a white solid with a melting point of 202-204 °C
 178 (Lit. 201-204 °C). The ^1H NMR revealed the presence of aromatic protons at δ 7.2, which
 179 integrated for two protons at positions 2 and 6 and that of methoxyl at δ 3.3 which integrated for
 180 3 protons at position 8. This indicated a simple aromatic system. The broad band was decoupled
 181 as; ^1H NMR: 7.20 (2H, H-2 and H-6), 5.0 (OH), 3.3 (3H, CH₃), ^{13}C NMR: 167.9 (C-7), 146.6
 182 (C-3), 146.6 (C-5), 139.4 (C-4), 122.2 (C-1), 110.3 (C-2), 110.3 (C-6), 52.5 (OMe). ^{13}C NMR
 183 revealed the presence of 8 carbons at δ (167.9 for one C=O, 110.3 for two CH, 52.5 for OCH and
 184 four quaternary aromatic carbons at 122.2, 138.4, 146.6, 146.6) (Table 1). HRMS of this fraction
 185 gave 184.03717 amu, which was consistent with molecular formula C₈H₈O₅. A library search

186 revealed it to be methyl gallate (methyl 3, 4, 5-trihydroxybenzoate) [15] and [24]. The sample
 187 was also compared with an authentic sample by TLC and was confirmed to be methyl gallate:
 188 Methyl 3, 4, 5-trihydroxybenzoate (Fig 3). Molecular formula; C₈H₈O₅

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190 Methyl gallate Figure 3. Chemical structure of methyl gallate with molecular formula C₈H₈O₅

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Table 1. Carbon 13 and Proton NMR spectra of methyl gallate (P1) and gallic acid (P2)

Position	Group	Compound P1		Compound P2	
		¹³ C Chemical Shift (δ _C)	Proton Chemical Shift (δ _H)	¹³ C Chemical Shift (δ _C)	Proton Chemical Shift (δ _H)
1	C	122.2	-	122.2	-
2	CH	110.3	7.2	110.3	7.0
3	C-OH	146.6	6.8	146.6	4.789
4	C-OH	139.4	5.0	140.0	4.789
5	C-OH	146.6	6.8	146.6	4.789
6	CH	110.3	7.2	110.3	7.0
7	C=O	167.9	-		
8	OCH ₃	52.5	3.30		

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196 The second compound (P2) was a solid, light brownish in colour, m. p. = 237-239°C. ¹H NMR:

197 7.0 (2H, H-2 and H-6), 4.789 (OH), ¹³C NMR: 170 (C-7), 146.6 (C-3), 146.6 (C-5), 139.4 (C-4),

198 122.2 (C-1), 110.3 (C-2), 110.3 (C-6). The ¹H NMR of this compound revealed the presence of

199 aromatic protons at δ7.0, which integrated for two protons at positions 2 and 6 and the OH

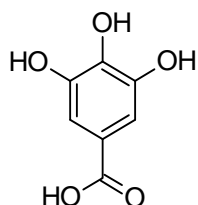
200 proton at position 7 at δ3.212. The broad band decoupled (Fig 4.) ¹³C NMR revealed a total of 7

201 carbons at δ (170 for one C=O, 110.3 for two CH, and 4 quaternary aromatic carbons at 122.2,

202 138.4, 146.6, 146.6). These were almost identical with that of methyl gallate except for the

203 absence of the OCH₃ ¹³C NMR peak. As a result this compound was subsequently identified as
 204 gallic acid [15]. Fig 4.

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207 Gallic acid
 208 Fig. 4 Chemical structure of gallic acid with molecular formula C₇H₆O₅

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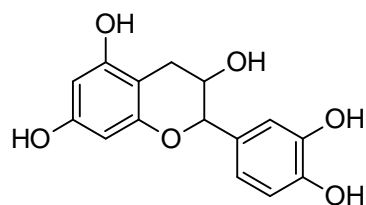
Table 2. Carbon 13 and Proton NMR spectra of Catechin (P3)

Position	Carbon 13 Chemical Shift (δ_C)	Proton Chemical Shift (δ_H)
1	-	
2	80.9	4.56
3	66.3	4.00
4	27.7	5.58
5	156.4	
6	93.9	
7		
8	95.1	
9		
1'		
2'	114	6.89
3'		
4'		
5'	115.1	6.79
6'	18.4	18.4

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 212
 213 ¹H-NMR spectra of the third compound (P3) showed peak at δ 4.56 (H-2, d), 4.01 (H-3, ddd),
 214 2.54 (H-4, dd), 2.90 (H-4, dd), 5.87 (H-6, d), 6.01 (H-8, d) 6.89 (H-2', d), 6.79 (H-5', d), 6.73
 215 (H-6', dd) and 8.00 (phenolic protons, m). ¹³C-NMR, Carbon atoms showed peaks at δ TMS 80.9
 216 (C-2), 66.3 (C-3), 27.7 (C-4), 93.9 (C-6), 95.1 (C-8), 114.5 (C-2'), 115.1 (C-5'), 18.4 (C-6')
 217 (Table 2). The NMR chemical shifts correlate well with those available in literature for catechin
 218 [25; 26]. The compound was identified as catechin (Fig 5).

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221

Catechin

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Fig 5. Chemical structure of catechin with molecular formula $C_{15}H_{14}O_6$

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3.2 Antibacterial activity

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Antibacterial activity of the crude fractions and compounds from fruit pulps of *A. nilotica* are as shown in table 3. The result shows that the crude extract was significantly active against all the test organisms but at varying degree of concentration. Minimum inhibitory concentration against *B. subtilis* and *S. aureus* were 500 and 750 ± 0.0 $\mu\text{g/ml}$ respectively while the susceptibility of Gram negative organisms (*E. coli*, *P. aeruginosa*, *S. typhi*, *K. pneumonia*) and *C. albicans* were slightly higher at 1000 – 2000 $\mu\text{g/ml}$ respectively). These concentrations are higher than the mic breakpoint for chloramphenicol (30 ± 0.1 $\mu\text{g/ml}$) the control drug. Hence the need for further purification of the extract to compound level for better activity. The fractions (F1-F6) produced by column chromatography with TLC finger printing yielded better minimum inhibitory concentrations (MICs) at 78 – 156 ± 0.0 $\mu\text{g/ml}$ respectively. Fraction F1 had no antibacterial effect, fraction F5 had mic of 156 $\mu\text{g/ml}$ against all the test organisms while F6 was only active against *S. aureus* and *B. subtilis* at 156 $\mu\text{g/ml}$. Fractions F2-F4 produced the best antibacterial effects at 78 $\mu\text{g/ml}$ against Gram positive organisms and 156 $\mu\text{g/ml}$ against Gram negative organisms. These fractions have a lot in common; TLC profile, positive test for phenolic and antibacterial effects. Microplate tetrazolium assay of isolated molecules against the

241 pathogenic organisms as shown in table 1, showed that methyl gallate, gallic acid at 39 $\mu\text{g/ml}$
242 and catechin at 19.5 $\mu\text{g/ml}$ concentration are significantly ($p < 0.05$) more effective against *E.*
243 *coli*, *S. aureus* and *B. subtilis* than against *P. aeruginosa*, *S. typhi* and *K. pneumonia* (78 $\mu\text{g/ml}$).
244 Catechin has shown to be the most effective of the molecules with MIC of 19 ± 0.5 $\mu\text{g/ml}$ against
245 *S. aureus* and *B. subtilis* and 39 $\mu\text{g/ml}$ against other Gram negative organisms.

246 This result shows that the purer the fractions the better the antibacterial activity. The use
247 of microplate tetrazolium assays to measure cell proliferation has increased exponentially since
248 their introduction [19]. Nevertheless, these assays do not actually measure the number of viable
249 cells in a culture or their growth but rather, an integrated set of enzyme activities that are related
250 in various ways to cell metabolism. They utilize the cofactor nicotinamide adenine dinucleotide
251 (NADH) and the dye 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium (MTT), and other
252 substrates like succinate and pyruvate which may also contribute to their reduction. Depending
253 on the particular dye chosen, reduction will be linked in various ways to cofactor/substrate
254 production, utilization and compartmentalization, and can be associated with the plasma
255 membrane, intracellular membranes, organelles and cytosol. Reduction can vary widely within
256 and between cell populations depending on the cell growth conditions, phase of cell growth and
257 stage of the cell cycle [20].

258 In the case of antibacterial activity of methyl gallate, the result is in agreement with a
259 similar study [27] that methyl gallate from the leaves of *Toona sureni* had antibacterial activity
260 against *E. coli*, *S. aureus* and *B. subtilis*. Other similar studies have reported that gallic acid by
261 mechanism of action in *P. aeruginosa*, *E. coli*, *S. aureus* and *Listeria monocytogens* led to
262 irreversible changes in membrane properties (charge, intra and extracellular permeability, and
263 physicochemical properties) through hydrophobicity changes, decrease of negative surface

264 charge, and occurrence of local rupture or pore formation in the cell membranes with consequent
 265 leakage of essential intracellular constituents [28]. Antibacterial activity of gallic acid of *A.*
 266 *nilotica* in this study corroborate the report that gallic acid inhibited the growth of
 267 *Campylobacter jejuni* and *E. coli* strains at 15.63-250 µg/ml. [12].

268 Prolonged incubation of the microplates was used to determine the end point assay of the
 269 molecules as bacteriocidal. Wells with bacteriostatic effect at concentrations lower than 78 µg
 270 /ml showed tetrazolium reduction after 48 hours of incubation while bacteriocidal concentrations
 271 of 78 µg /ml upward remained colourless. This assay has been used to distinguish between
 272 dormant and metabolically active microbial cells (Tab. 3). The test organisms were able to
 273 reduce tetrazolium dyes in their electron transport chain, generating results within hours.

Molecules µg/ml	MICROORGANISMS						
	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. typhi</i>	<i>K. pneumo nia</i>	<i>Ca</i>
Crude extract	750 ± 0.0	1000 ± 0.0	1000 ± 0.0	500 ± 0.0	2000 ± 0.0	2000 ± 0.0	1000 ± 0.0
F1	NA	NA	NA	NA	NA	NA	NA
F2	78	156	156	78	156	156	156
F3	78	156	156	78	156	156	156
F4	78	156	156	78	156	156	156
F5	156	156	156	156	156	156	156
F6	156	NA	NA	156	NA	NA	156
Methyl gallate	39 ± 0.0	*78 ± 0.0	78 ± 0.0	39 ± 0.0	78	78	39
Gallic acid	39 ± 1.0	78 ± 0.0	78 ± 0.0	39 ± 0.0	78 ± 0.0	78 ± 0.0	39 ± 0.0
Catechin	19.5 ± 0.0	39 ± 0.0	39 ± 0.0	19.5 ± 0.0	39 ± 0.0	39 ± 0.0	39 ± 0.0

Chloramphenicol	30± 0.0	30 ± 0.00	30 ± 0.0	30±.0.0	30±0.0	30±0.0	
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275 **Table 3. Antibacterial activity of crude, fractions and compounds of fruit pulp of *A. nilotica***

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277 **Key:** F= Fractions, NA = No activity, no growth inhibition

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279 **3.3 Time kill kinetic antibacterial of crude extract**

280 The time kill kinetic antibacterial of crude extract against *S. aureus* and *E. coli* are as
 281 shown in Figs. 6 and 7. The percentage of growth reduction in *S. aureus* at 2, 4, 6, 8 and 12 hrs
 282 of contact were 52.2, 60.8, 70, 80 and 99.2% respectively. Percentages of *E.coli* growth
 283 reduction for each corresponding time were; 45.7, 51.7, 64.16 and 99.9% respectively. The
 284 extent and rate of killing of the organism by the extract at 2 x MIC followed the same trend as
 285 the concentrations of the extract increased with increase in contact time, the percentage of the
 286 organisms killed also increased.



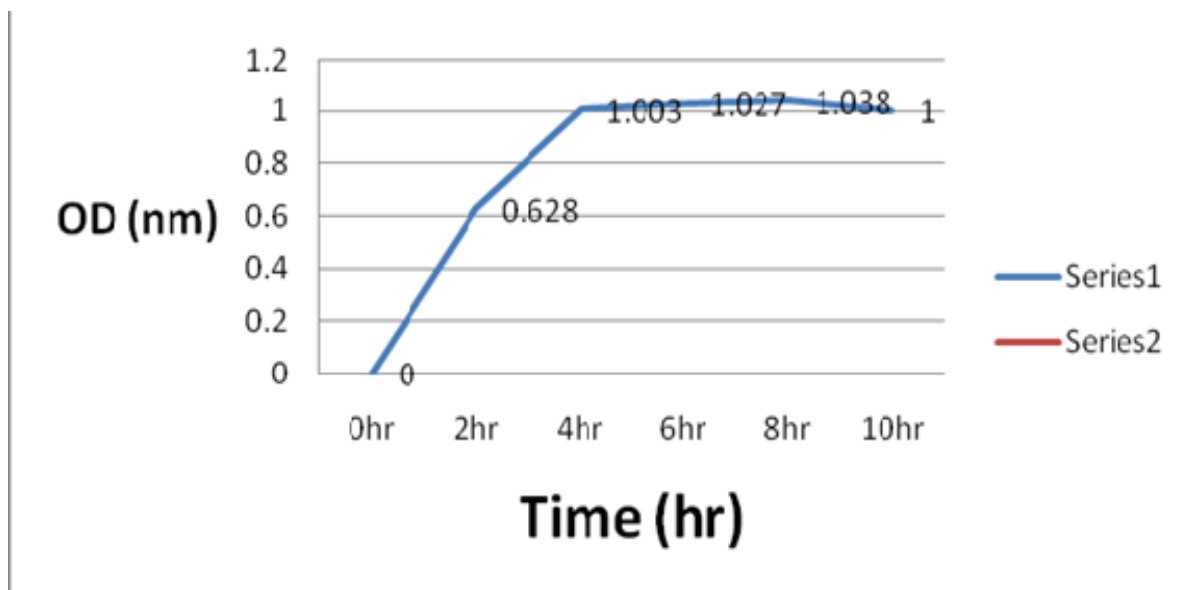
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294 **Fig. 6. Effect of extract of *A. nilotica* on the growth of *S. aureus* at different time**

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297 **Fig 7. Effect of extract of *A. nilotica* on the growth of *E. coli* at different time**

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301 **4.0 Conclusion**

302 In conclusion, this study has demonstrated the antibacterial properties of bioflavonoids
 303 from fruit pulps of *A. nilotica*. The compounds isolated showed an improved antibacterial effects
 304 justifying the need for high-through put isolation techniques in plant research. The time kill
 305 antibacterial study was able to demonstrate time dependent effect of the crude extract against the
 306 test organisms. Generally, the compounds isolated could serve as leads in the search for new and
 307 potent antibacterial compounds from indigenous medicinal plants.

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310 **Conflict of interest:** Authors have declared that no competing interests exist

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