Antibacterial Activity of Phytochemicals in *Ficus thonningii* Leaves Extracts Against Some Selected Pathogenic Bacterial Prevalent in Sickle Cell Anemia

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**ABSTRACT**

Sickle cell anemia (SCA) is caused by point mutation involving substitution of valine by glutamic acid, clinical cause of this is that SCA patients are immunocompromised hence, prone to bacterial infections. *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Staphylococcus pneumoniae* and *Staphylococcus aureus* are some of the bacterial associated with SCA. Here we investigated the antibacterial activity of extracts of *Ficus thonningii* leaves used by ethnomedicinal practitioners in the management of infections in SCA patients. The antimicrobial activity was determined based on average diameter of zone of inhibition (AVDZI), minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) while, structure of compounds FTH1 (Bergapten), FTH2 (Protocatechuic acid) and FTH3 (Methyl ferulate) were identified based on Nuclear Magnetic Resonance (NMR) analysis. The results indicated that the crude extracts of methanol (MCE), hexane (HCE), chloroform (CCE) and methanol-water (MWCE) had AVDZI of ≥ 12.1 ± 0.12 mm, ≥ 11.0 ± 0.00 mm, ≥ 10.2 ± 0.25 mm and ≥ 12.1 ± 0.21 mm respectively while the isolated compounds FTH1, FTH2 and FTH3 had AVDZI of ≥ 10.1 ± 0.10 mm, ≥ 9.1 ± 0.12 mm, ≥ 7.1 ± 0.24 mm respectively. The AVDZI results was tested for statistical significance using one way ANOVA and Tukey Posthoc test, and was considered significant at p < 0.05. Our findings suggested that *F. thonningii* leaves extracts including compounds isolated from them are potential antibacterial agent and justify their use as antibacterial prophylactics in the management of infections in SCA patients by ethnic people in Nigeria.

**Keywords**: Antibacterial, *Ficus thonningii*, infections, sickle cell anemia, ethnomedicine.

1. **INTRODUCTION**

Infections in sickle cell anemia are caused by several factors and pathological effects of sickle cell anemia (SCA) most times create an environment that support infections . Area of necrotic bones act as foci for infections in SCA patients, which becomes established through hematogenous spread . Generally, people living with SCA are immunocompromised however, the disease shows different level of severity and manifestations in different patients at different stages owing to patient phenotype . Also, SCA patients are predisposed to certain iatrogenic infections due to therapeutic interventions . Infections has long been known as one of the causes of vaso-occlusive crisis because they promote leukocyte and Red Blood Cell (RBC) adhesion . Infections can possess more non-specific effects that increases the risk and likelihood of sickling, this includes fever with water loss due to sweating, anorexia, and nausea with reduced oral fluid intake, diarrhea and vomiting contributes to dehydration .

They exist a correlation between respiratory infection and acute chest syndrome . Research indicates that during infection changes occurs at cellular level that predisposes SCA patients to crisis, this change occurs locally and systematically in infected tissues, such that...
neutrophils, basophils and monocytes attracted to sites of inflammations produces cytotoxic proteins which generates reactive oxygen species that causes oxidative damage, which then promotes endothelial activation and cell adhesion. Sickle cell anemia is prevalent among Black Africans, Afro-Caribbean, Mediterranean, middle east and some parts of India and infections contributes significantly to mortality and morbidity in SCA. Salmonella spp., Staphylococcus spp., K. pneumoniae, E. coli, Enterobacter spp., Acinetobacter spp., Streptococcus spp., Serratia spp., are among prevalent bacteria implicated in SCA responsible for arrays of infections among which includes but not limited to septicemias, urinary tract infections, myositis, meningitis etc. The use of synthetic antibiotic prophylaxis though beneficial; poses risk on SCA patient thus, the use of antibiotic prophylaxis attempt to strike a balance between risk to the individual and the danger resistant organisms pose to the whole population. Research indicates that 9% of SCA patients with S. pneumoniae infection had reduced susceptibility to penicillin however, in those on prophylaxis, research suggest the rates may be much higher. Therefore, it is necessary to evaluate natural products as possible alternative in prophylactic management of SCA patients since mostly third world countries bears the burden of SCA.

Although, the antibacterial activity of Ficus thonningii leaves as well as some of the compounds isolated from them have been reported. However, in this study we profiled methanol (MCE), chloroform (CCE), methanol-water (MWCE) and n-hexane (HCE) crude extracts as well as compounds isolated from CCE and MWCE on E. coli, K. pneumoniae, S. typhi, S. pneumoniae and S. aureus isolated from SCA patients in vitro to understand the medicinal bases for its use as antibacterial prophylaxis by ethnomedicine practitioners in the management and treatment of infections in SCA patients in Ebonyi State, Nigeria.

2. MATERIALS AND METHODS

2.1 Sample collection, identification and preparation

The leaves of Ficus thonningii used in this study were collected from Echi-Aba in Echi-Aba Development Center, Ebonyi Local Government Area Council, Nigeria (latitude: 6°24’51.9”N, longitude: 8°07’34.1”E). The plant locally known by the ethnic people as Orgbu was identified and authenticated by a taxonomist at the Department of Applied Biology, Ebonyi State University as Ficus thonningii. The leaves were air dried and pulverized with the aid of both mechanical grinder and mortar then kept for further use in an air tight container. All chemicals used were Sigma Aldrich quality grade, melting point was determined using a Duran Thiele apparatus while the structure of isolated compound was determined using Bruker 500MHz spectroscopy. All bacteria used were local clinical isolates while the ampicillin used was Cikacillin© (Ampicillin Trihydrate BP 250 mg). Statistical (Tukey posthoc) analysis was done in triplicate using SPSS software version 20 and regarded as significant at P < 0.05. The results of statistical analysis were presented as mean ± standard error of the mean (SEM)

2.2 Extraction and purification

Sequential extraction of the plant part was successively carried out separately with solvents of increasing polarity: n-hexane, chloroform, methanol and methanol-water mixture (4:1). Pulverized leaves (10 kg) was weighed out and soaked in the appropriate solvents in order of increasing polarity for 72 hours. The mixture was filtered, the filtrate was concentrated using a rotary evaporator (Stuart RE 300/MS, UK) to one-tenth of its volume at ≤ 40°C. Each dried extract was weighed in an analytical balance (OHAUS PX225D, USA) and stored at 4°C.

2.3 Column and flash chromatographic separation

In each, 15 g of the crude extract was subjected to column chromatography and eluted with Hex- EtOAc (80:20, 70:30, 60:40, 50:50), EtOAc (100%) and MeOH (100%) gradients. Slurry of silica gel 70-230 mesh (600 g)
was made with the eluting solvent and packed into the glass column. The tap was opened to allow excess solvent to drain off. The leaves extracts (15 g) was dissolved in the eluting solvent and packed on top of the silica gel slurry. As soon as the column began to condition, glass wool fiber was placed on top of the extract and the eluting solvent was added. Collection of the eluent was done with 50 mL and 100 mL conical flasks. Further elution was done with increasing concentration gradients. For the MeOH leaves crude extract, elution was carried out using DCM-EtOAc (80:20, 70:30), EtOAc (100%), EtOAc-MeOH (50:50) and MeOH (100%) gradients. For n-Hex leaves crude extract, elution was done with Hex-DCM gradients (60:40, 50:50), EtOAc (100%), EtOAc-MeOH (50:50), and MeOH (100%) gradients. For CHCl₃ leaves crude extract, elution was done with Hex-DCM gradients (60:40, 50:50), EtOAc (100%), EtOAc-MeOH (50:50), and finally with 100% MeOH. Elution of MeOH-H₂O leaves extract was carried out with DCM-EtOAc (80:20), EtOAc (100%), MeOH (100%). Fractions collected were monitored for purity by spotting on thin layer chromatographic (TLC) plates. The fractions were subjected to further separation and purification using column and flash chromatographic technique. The fractions in CHCl₃ leaves fraction were FTH1, and FTH2 whereas, FTH3 was extracted from the MeOH-H₂O. Collected fractions from column chromatography were further purified using a solvent system of PET-CHCl₃ (4:1) as the mobile phase and this revealed one major spot with minor spots, these fractions were further purified using flash chromatographic technique (silica gel, mesh 230-400, 30 g) prior to each NMR analysis. Elution was initially carried out with varying solvent mixture of PET-CHCl₃. Elution with solvent mixture of PET-CHCl₃ (5:1) yielded a major single spot on TLC with some minor impurities at the origin. The process was repeated and similar for all fractions. Concentration, drying and washing of the fractions severally with methanol afforded extracts labelled FTH1, FTH2 and FTH3. The pure fractions were further recrystallized three times, weighed and stored for use at 4°C

2.4 Nuclear Magnetic Resonance

Both the 1D and 2D NMR spectral analyses were carried out using Bruker 500MHz NMR spectrometer. The samples were dissolved in the appropriate solvent prior to analysis. The structures were elucidated using a combined NMR technique of ¹H, ¹³C, DEPT-135, TOCSY, COSY, HSQC and HMBC NMR

2.5 Evaluation of antimicrobial activity

2.5.1 Determination of diameter of zone of inhibition

Bacteria organisms were obtained from Alex Ekwueme Federal University Teaching Hospital Abakaliki. The bacteria isolates were tested for viability by resuscitating them in buffered peptone water afterward they were subcultured into nutrient agar medium and incubated at 37°C for 24 hours. The organisms were then stored at 4°C until when needed. Agar well diffusion techniques as described by Adeniyi et al. was adopted for the study. 18 mL of Mueller Hinton agar (MHA) plates (Oxoid, England) were inoculated with 0.1 mL of an overnight broth culture of each clinical bacteria isolate (Equivalent to 3 x 10⁷ cfu/mL) McFarland (MF) standard in sterile petri-dish. The seeded plates were rocked for uniform distribution of the bacteria isolates and allowed to set. Holes were bored on the plates using standard sterile cork borer of 6 mm diameters and equal volumes of the proposed antimicrobial agent (1000 µL) were transferred into the well with the aid of micropipette. The experiments were done in triplicate. The control experiments were setup with 1000 µL of 70% methanol in separate well. The plates were allowed to stand for one hour at room temperature to allow proper diffusion of the extract. The plates were incubated at 37°C for 24 hours until marked decline in potency of antibacterial agent to inhibit the growth of the test bacteria was observed. Zone of inhibitions were measured in millimeter (mm) and the average values were calculated and recorded.
2.5.2 Determination of the minimum inhibitory concentration

The method described by Adebayo et al. 18 was used. The determination of the minimum inhibitory concentration (MIC) was carried out on extracts and bacteria isolates that showed sensitivity against the growth of the test organisms. The medium used was MHA solution which was prepared according to the manufacturer’s standard of 38 g/1000 mL. In this study double strength was prepared by dissolving 38 g in 500 mL of distilled water, homogenized and 5 mL was dispensed into 40 sets of McCartney bottles and sterilized in an autoclave at 121°C for 15 min. The agar was allowed to cool to approximately 45°C and each graded solution was then poured into Petri-dishes and allowed to solidify for one hour. Extracts concentration of 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.125 mg/mL were prepared by serial dilution. Each plate was divided into 4 (four) equal sections and labeled accordingly. The 5 mm diameter paper discs were placed aseptically into each labeled section of the plate using sterilized forceps. With an automatic micropipette, 0.1 mL of each bacterial suspension was taken and transferred aseptically into each appropriate pre-labeled paper disc on the agar plates. The plates were incubated for 24 hours at 37°C after which they were observed for growth or death of the test organisms. The lowest concentration inhibiting growth was taken as the minimum inhibitory concentration (MIC).

2.5.3 Determination of the minimum bactericidal concentration (MBC)

The determination of MBC was carried out by preparing 40 sets of plates of Mueller Hinton agar and sterilized. The paper discs in all the plates from MIC tests were reactivated, using a mixture of 0.5% egg lecithin and 3% Tween 80 solution. The reactivated organisms were subcultured into appropriately labeled quadrants of the sterilized Mueller Hinton agar plates. The organisms were uniformly streaked on labeled quadrants using wire loop. The organisms were incubated at 37°C for 24 hours, after which growth were observed and recorded. The MBC was the quadrant with lowest concentration of the extract without growth.

3. RESULTS AND DISCUSSIONS

3.1 Results of structural elucidations

FTH1: - Bergapten

*White crystalline solid; Rₐ 0.68; melting point 188-190°C 19; yield 0.13 %.* ¹³C (500MHZ, CDCl₃): 161.30 ppm (C-8), 158.36 ppm (C-11), 152.67 ppm (C-9), 105.09 ppm (C-1), 144.79 ppm (C-2), 149.55 ppm (C-4), 139.33 ppm (C-6), 112.58 ppm (C-3), 112.48 ppm (C-7), 106.32 ppm (C-5), 93.78 ppm(C-10), 60.06 ppm (C-12). ¹H (500MHZ, CDCl₃): 8.16 ppm (H-6), 7.59 ppm (H-2), 7.13 ppm (H-1), 6.28 ppm (H-7), 4.27 ppm (H-12). The ¹⁳C, ¹H, DEPT-135, TOCSY, COSY, HSQC and HMBC spectra for FTH1 including its physical characterizations matched those of Bergapten and was thus assigned 20, 21.

![Structure of Bergapten](image)

**Figure 1. This figure depicts structure of Bergapten isolated from *F. thonningii* leaves**

FTH2: - Protocatechuic acid

*Gray crystalline solid; Rₐ 0.72; melting point 221-223°C 22; yield 0.16 %.* ¹³C (500 MHZ, D₂O): 177.94 ppm (C-7), 150.04 ppm (C-3), 146.07 ppm (C-4), 131.39 ppm (C-5), 125.24 ppm (C-1), 119.63 ppm (C-2), 118.12 ppm (C-6). ¹H (500 MHZ, D₂O): 7.42 ppm (H-2), 7.39 ppm (H-6), 6.92 ppm (H-5). The 13C, 1H, DEPT-135, TOCSY,
COSY, HSQC and HMBC spectra including physical characterizations for FTH2 matched those of protocatechuic acid and was thus assigned.

Figure 2. This figure depicts structure of protocatechuic acid isolated from *F. thonningii* leaves.

**FTH3:** - Methyl ferulate

*Yellowish brown crystal,* Rf: 0.83, melting range: 63 - 65 °C, mass: 14.68 g yield 0.15%. 13C (500MHZ, DMSO-d6): 167.09 (C-9), 149.36 (C-4), 147.91 (C-3), 145.10 (C-7), 125.55 (C-1), 123.12 (C-6), 115.50 (C-8), 114.19 (C-5), 111.29 (C-2), 55.71 (3-OCH3), 51.22 (9-OCH3). 1H (500MHZ, DMSO-d6): 7.57 (H-7), 7.32 (H-6), 7.12 (H-2), 6.80 (H-5), 6.50 (H-8), 6.46 (4-OH), 3.81 (3-OCH3), 3.70 (9-OCH3). The 13C, 1H, DEPT-135, TOCSY, COSY, HSQC and HMBC spectra for FTH3 including its physical characterizations matched those of methyl ferulate and was thus assigned.

Figure 3. Methyl ferulate isolated from *F. thonningii* leaves.

3.2 Results of Average diameter zone of inhibition

<table>
<thead>
<tr>
<th>Extracts, and Drug</th>
<th><em>Escherichia coli</em></th>
<th><em>Klebsiella pneumonia</em></th>
<th><em>Salmonella typhi</em></th>
<th><em>Streptococcus pneumoniae</em></th>
<th><em>Staphylococcus aureus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>MCE</td>
<td>16.3±0.30a</td>
<td>20.0±0.00a</td>
<td>12.1±0.12a</td>
<td>20.1±0.21a</td>
<td>18.0±0.15a</td>
</tr>
<tr>
<td>HCE</td>
<td>13.1±0.15b</td>
<td>19.2±0.20b</td>
<td>11.0±0.00b</td>
<td>20.3±0.23a</td>
<td>16.2±0.31b</td>
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<tr>
<td>CCE</td>
<td>12.3±0.15c</td>
<td>14.2±0.20c</td>
<td>10.2±0.25c</td>
<td>18.3±0.16c</td>
<td>15.1±0.13c</td>
</tr>
<tr>
<td>MWCE</td>
<td>14.1±0.10d</td>
<td>17.0±0.00e</td>
<td>12.1±0.21a</td>
<td>18.1±0.10f</td>
<td>18.2±0.23a</td>
</tr>
<tr>
<td>FTH1</td>
<td>10.1±0.10e</td>
<td>13.2±0.10e</td>
<td>11.0±0.00b</td>
<td>20.1±0.10a</td>
<td>13.0±0.00e</td>
</tr>
<tr>
<td>FTH2</td>
<td>9.1±0.12f</td>
<td>12.1±0.13f</td>
<td>10.2±0.09c</td>
<td>15.1±0.18f</td>
<td>12.1±0.21e</td>
</tr>
<tr>
<td>FTH3</td>
<td>9.0±0.00f</td>
<td>11.2±0.20f</td>
<td>10.0±0.00c</td>
<td>13.1±0.19g</td>
<td>7.1±0.24f</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>19.1±0.12h</td>
<td>23.0±0.09h</td>
<td>17.2±0.17h</td>
<td>28.1±0.12h</td>
<td>25.2±0.17h</td>
</tr>
</tbody>
</table>

* a, b, c, d, e, f, g and h indicate the level of significance of the difference between different agents and Ampicillin as obtained from SPSS statistics Tukey Posthoc test. Distinct letters in the same column indicates significant difference (P < 0.05) while two letters of the same identity indicate non-significant difference (P > 0.05). MCE = methanol crude extract; HCE = hexane crude extract; CCE = chloroform crude extract; MWCE = methanol-water crude extract; FTH1 = bergapten; FTH2 = protocatechuic acid; FTH3 = methyl ferulate.
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Average diameter of zone of inhibition was used to evaluate the antibacterial activity of the antibacterial drug candidates and ampicillin. The results were presented as mean ± SEM. From Table 1, it was shown that the extracts and isolated compounds of *F. thonningii* showed viable activity against both gram positive (*S. aureus* and *S. pneumoniae*) and gram negative (*K. pneumonia, E. coli* and *S. typhi*) bacteria used in the study. The antibacterial activity of both the isolated compound and the crude extracts were significantly different from those of ampicillin at P < 0.05 i.e. the inhibitory activity of each antibacterial agent was significantly different from that of ampicillin. Consequently, bacteria used in the study were susceptible to both the antimicrobial drug candidate and ampicillin.

### 3.3 Results of Minimum inhibitory concentration

**Table 2. Minimum inhibitory concentration of *F. thonningii* leaves extracts (mg/mL)**

<table>
<thead>
<tr>
<th>Extracts, and Drug</th>
<th><em>Escherichia coli</em></th>
<th><em>Klebsiella pneumoniae</em></th>
<th><em>Salmonella typhi</em></th>
<th><em>Streptococcus pneumoniae</em></th>
<th><em>Staphylococcus aureus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>MCE</td>
<td>1.000</td>
<td>0.250</td>
<td>4.000</td>
<td>4.000</td>
<td>2.000</td>
</tr>
<tr>
<td>HCE</td>
<td>4.000</td>
<td>1.000</td>
<td>8.000</td>
<td>2.000</td>
<td>8.000</td>
</tr>
<tr>
<td>CCE</td>
<td>8.000</td>
<td>4.000</td>
<td>8.000</td>
<td>8.000</td>
<td>16.000</td>
</tr>
<tr>
<td>MWCE</td>
<td>2.000</td>
<td>2.000</td>
<td>4.000</td>
<td>8.000</td>
<td>4.000</td>
</tr>
<tr>
<td>FTH1</td>
<td>16.000</td>
<td>8.000</td>
<td>8.000</td>
<td>4.000</td>
<td>16.000</td>
</tr>
<tr>
<td>FTH2</td>
<td>32.000</td>
<td>16.000</td>
<td>8.000</td>
<td>32.000</td>
<td>16.000</td>
</tr>
<tr>
<td>FTH3</td>
<td>32.000</td>
<td>32.000</td>
<td>32.000</td>
<td>32.000</td>
<td>64.000</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>0.250</td>
<td>0.500</td>
<td>1.000</td>
<td>0.250</td>
<td>0.125</td>
</tr>
</tbody>
</table>

MCE = methanol crude extract; HCE = hexane crude extract; CCE = chloroform crude extract; MWCE = methanol-water crude extract; FTH1 = bergapten; FTH2 = protocatechuic acid; FTH3 = methyl ferulate

From Table 2, with respect to *E. coli* the MIC of MCE (1.000 mg/mL), HCE (4.000 mg/mL), CCE (8.000 mg/mL) and MWCE (2.000 mg/mL) were slightly comparable to ampicillin (0.250 mg/mL). Similarly, it was observed that MCE inhibited the growth of *K. pneumoniae, S. typhi, S. pneumoniae, and S. aureus* at 0.250 mg/mL, 4.000 mg/mL, 4.000 mg/mL and 2.000 mg/mL concentrations respectively. Comparatively FTH3 had the highest minimum inhibitory concentration values for all analyzed bacteria indicating high dose requirements in vitro. MCE, HCE and MWCE inhibited the growth of *K. pneumoniae* at lower concentrations comparable to ampicillin.

Patients with homozygous sickle cell (SS) disease are at increased risk of infection with *Streptococcus pneumoniae, Haemophilus influenzae* type b, *Salmonella* spp, *Escherichia*
coli and Klebsiella spp \(^{33, 34, 35}\) therefore, the ability of the extracts of \(F. thonningii\) and its isolated compounds to inhibit the growth of \(E. coli\), \(K. Pneumoniae\), \(S. typhi\) and \(S. pneumoniae\) even at lower concentrations validated the use of \(F. thonningii\) in the management of infections in SCA patients in Southeast Nigeria.

3.4 Results of Minimum Bactericidal concentration

<table>
<thead>
<tr>
<th>Extracts, and Drug</th>
<th>\textit{Escherichia coli}</th>
<th>\textit{Klebsiella pneumonia}</th>
<th>\textit{Salmonella typhi}</th>
<th>\textit{Streptococcus pneumoniae}</th>
<th>\textit{Staphylococcus aureus}</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCE</td>
<td>2.000</td>
<td>1.000</td>
<td>8.000</td>
<td>8.000</td>
<td>2.000</td>
</tr>
<tr>
<td>HCE</td>
<td>8.000</td>
<td>2.000</td>
<td>16.000</td>
<td>4.000</td>
<td>16.000</td>
</tr>
<tr>
<td>CCE</td>
<td>16.000</td>
<td>8.000</td>
<td>16.000</td>
<td>16.000</td>
<td>32.000</td>
</tr>
<tr>
<td>MWCE</td>
<td>4.000</td>
<td>4.000</td>
<td>8.000</td>
<td>16.000</td>
<td>4.000</td>
</tr>
<tr>
<td>FTH1</td>
<td>32.000</td>
<td>16.000</td>
<td>16.000</td>
<td>8.000</td>
<td>32.000</td>
</tr>
<tr>
<td>FTH2</td>
<td>64.000</td>
<td>32.000</td>
<td>16.000</td>
<td>64.000</td>
<td>32.000</td>
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<tr>
<td>FTH3</td>
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<td>64.000</td>
<td>NA</td>
</tr>
<tr>
<td>Ampicillin</td>
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<td>1.000</td>
<td>2.000</td>
<td>1.000</td>
<td>0.250</td>
</tr>
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</table>

\(\text{NA} = \) No activity at coverage concentration; \(\text{MCE} = \) methanol crude extract; \(\text{HCE} = \) hexane crude extract; \(\text{CCE} = \) chloroform crude extract; \(\text{MWCE} = \) methanol-water crude extract; \(\text{FTH1} = \) bergapten; \(\text{FTH2} = \) protocatechuic acid; \(\text{FTH3} = \) methyl ferulate

The minimum bactericidal concentration is the lowest concentration of an antimicrobial agent required to kill a bacterium \(^{36}\). The MBC demonstrates the lowest level of antimicrobial agent that results in microbial death. Antibacterial agents are usually regarded as bactericidal if the MBC is no more than four times the MIC \(^{37}\). From Table 3, it was shown that the MCE had the lowest MBC values when compared with other extracts of \(F. thonningii\) this was followed by MWCE. Methanol as an extracting solvent has high extractability and its polarity works on many phytochemicals including polar and nonpolar Phyto-
constituents. FTH1 and FTH2 were compounds isolated from CCE of \(F. thonningii\) while FTH3 was identified from its methanol-water extract. Therefore, it is expected that the improved MBC (antibacterial activity) of MCE was probably due to compounds contained in its methanol and methanol-water extracts that function synergistically with other phytocompounds toward improved antibacterial activity.

The Burden and spectrum of bacterial infections among children living with SCA suggest that \(Salmonella\) spp., \(Staphylococcus\) spp., \(K. pneumoniae\), \(E. coli\), \(Enterobacter\) spp., \(Acinetobacter\) spp., \(Streptococcus\) spp., \(Serratia\) spp., were responsible for 28.1%, 18.8%, 17.7%, 10.4%, 5.2%, 4.2%, 4.2%, and 4.2% of infections respectively and most cases of septicemias were reportedly caused by \(Staphylococcus\) spp. (24.6%), \(Salmonella\) spp. (24.6%) and \(Klebsiella pneumoniae\) (16.9%) also, \(E. coli\) led to majority of cases of urinary tract infections (53.8%) and \(Salmonella\) spp. were responsible for all cases of myositis while \(S. pneumoniae\) was responsible for two cases of meningitis \(^{38}\). Since previous report suggest that ethnomedicinal plant-based antibacterial agent are potent in the treatment of infections caused by \(E. coli\), \(S. aureus\) and \(S. pneumoniae\) and several study support its broad-spectrum antibacterial activity \(^{39, 40}\) hence, indicating the efficacy of ethnomedicine in the treatment of infections. Therefore, these bacteria even at very low extract
concentrations were susceptible to *F. thonningii* leaves hence, the use of *F. thonningii* leaves extract as a natural product prophylactic may possibly reduce the occurrence of these infections in SCA patients and thus possibly may also ameliorate the condition of sickle cell patients with respect to recurrence of painful episodes that occurs due to bacterial infections

**CONCLUSION AND RECOMMENDATIONS**

The antibacterial activity of the leaves extracts of *F. thonningii* was profiled against *E. coli*, *K. pneumoniae*, *S. typhi*, *S. pneumoniae* and *S. aureus* because of the prevalence of these bacterial and its infections among people living with SCA. The observed diameter of zone of inhibition showed that the crude extracts of the plant parts and compounds isolated from them showed average diameter of zone of inhibition comparable to those of ampicillin while the analyses of the MIC and MBC showed that the extracts inhibited the growth of these bacteria even at low concentrations thus, supporting the ethnomedicinal use of this plant in the treatment of infections in children living with SCA. Hence, *F. thonningii* should further be profiled in vivo for use in the management of infections in SCA patients. Clinical trials of compounds isolated from *F. thonningii* including those reported herein should be considered both as antibiotic-enhancer and prophylactics in the management of infections in SCA patients.

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**Competing interest disclaimer**

The authors declare that no competing interests exist, and the research received no funding.

**REFERENCES**


Antibacterial Activity of ...


النشاط المضاد للبكتيريا للمواد الكيميائية النباتية في أوراق Ficus thonningii المستخلصات ضد بعض البكتيريا المسببة للأمراض المنتشرة في فقر الدم المنجلي

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ملخص
تتحدث فقر الدم المنجلي عن طفرة نقطة تتضمن استبدال حمض الغلوتاميك بالفالين، وبالتالي يكونون عرضاً للعديد البكتيريا. الإشريكية القولونية، كليبسيلا، الرئة، السالمونيلا التيفية، العقدية الرئوية والكوارتلا الذهبية هي بعض البكتيريا المرتبطة بـ SCA. هنا قمنا بالتحقيق في النشاط المضاد للبكتيريا لمستخلصات أوراق Ficus thonningii التي يستخدمها ممارس الطب العرقي في إدارة العدوى في مرضى SCA. تم تحديد النشاط المضاد للميكروبات بناءً على متوسط قطر منطقة التثبيط (AVDZI) والتركيز المثبط الأدنى (MIC) والحد الأدنى لتركيز مبيد الجراثيم (MBC) بينما، بنية المركبات (FTH1) و (FTH2) و (FTH3) (Protocatechucetic، Bergapten) بناءً على تحليل الرنين المغناطيسي (NMR) أشارت النتائج إلى أن المستخلصات الخم ميثانول (MCE) والهكسان (HCE) والكلوروفورم (CCE) تحتوي على AVDZI وميثانول الماء (MWCE) يحتوي على 12.1 ± 0.21 مم على التوالي. تم اختبار نتائج من أجل الفرق بين ABZI بمعدل 0.00 ± 0.12 مم، ≤ 10.2 مم على التوالي مباشرة. استخدمت اختبار ANOVA المدى الإحصائي باستخدام اختبار Tukey Posthoc عند p < 0.05. تم اختبار النتائج التي توصلنا إليها إلى أن مستخلصات أوراق F. thonningii و F. thonningii من خلال اختبار التجارب حاملة وقائي مضاد للبكتيريا في إدارة العدوى في مرضى SCA من قبل الأشخاص العرقيين في نيجيريا.

الكلمات الدلالة: مضاد للجراثيم، اللبخ thonningii، الالتهابات، فقر الدم المنجلي، الطب العرقي.

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