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The Effect of *Acacia nilotica* on Helicobacter pylori Colonization

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Abstract: *The biological activity and the effects of aqueous Acacia nilotica leaf extracts on H. pylori colonization factors were investigated. The extracts were assessed for antimicrobial, anti-urease and anti-adhesion properties against H. pylori using a combination of in vitro, in situ, and in vivo assays. Acacia nilotica showed moderate antimicrobial activity against H. pylori with a minimum inhibitory concentration and minimum bactericidal concentration of 180 µg/mL and 5 mg/mL respectively. The total phenolic and flavonoid contents in Acacia nilotica extract were 154.0 mg gallic acid equivalent (GAE)/g and 8 mg quercetin equivalent (QE)/g of dry extract. The extract also showed a high antioxidant activity of 312 mg ascorbic acid equivalent/g extract. GC-MS extract analysis showed 2-Cyclohexen-1-one-4,4-d2, 6,6-dimethyl- (28.10%), 2,5,5-trimethyl-1-cyclopent-2-enon (14.00%) and -2-methyl(3-D) propenoic acid (9.42%) as the major constituents. The extract also showed high anti-urease activity and high anti-adhesion property both in vitro and in vivo. The results of this study showed that Acacia nilotica can affect the colonization of H. pylori in the stomach, and can be further explored for its pharmaceutical application for the H. pylori eradication.*

Keywords: *Acacia nilotica; phytomedicine; Helicobacter pylori; colonization factors; anti-ureasic; anti-adhesion*

Abbreviations

CFU, Colony Forming Unit;
ELISA, Enzyme Linked Immunosorbent Assay;
FITC, fluorescein isothiocyanate;
MBC, Minimum Bactericidal Concentration;
MIC, Minimum Inhibitory Concentration;
PBS, Phosphate Buffered Saline;
PBST, Phosphate Buffered Saline and Tween 20;
TRITC, Tetramethylrhodamine;
FISH, Fluorescent *in situ* Hybridization
GC-MS, gas Chromatography – Mass Spectrometry

1. INTRODUCTION

Infection with *Helicobacter pylori* affects half of the world's population, and its distribution is influenced by geographical location, ethnicity, socioeconomic factors, age, and gender [1,2]. Three steps are required for *H. pylori* colonization in the stomach [3]. These are: (1) survival in the acidic conditions of the stomach; (2) migration towards epithelial cells via flagella-mediated motility; and (3) adhesin attachment to host receptors. These steps are known as *H. pylori* virulence/colonization factors. The inability of *H. pylori* to express its colonization factors makes it vulnerable to the host defense system, potentially leading to the bacteria's eradication in the stomach.

Currently, multiple antibiotics are used to treat *H. pylori* infection. However, multiple resistance to these antibiotics in Nigeria necessitates the identification of alternative therapies for the adjuvant treatment of *H. pylori* [4,5]. Such ideal therapy with anti-*Helicobacter pylori* activity should be capable of counteracting one or more of *H. pylori*'s virulence/colonization factors, preventing its survival in the stomach lining.

Acacia nilotica (L.) Delile, also known as Acacia arabica (Lam.) Willd. (Mimosaceae), is a tropical and subtropical tree. *Acacia nilotica* has high potential antioxidant activity and has traditionally been used to treat a variety of ailments [6]. *Acacia nilotica* grows in northern Nigeria, where it is used to treat gastrointestinal ailments. An ethnopharmacological study in Southern and North Central Nigeria reported the use of *Acacia nilotica* seeds and leaves for the treatment of gastric ulcers and other gastrointestinal ailments [7]. However, there is a lack of data on the plant's anti-*H.pylori* activity. As a result, it is necessary to confirm whether the plant can be used to treat *H. pylori* infection (which is a major cause of gastrointestinal disorders).

Studies have reported that therapies that interfere with bacterial adhesion can be used to prevent and treat bacterial infections [8]. As a result, any proposed adjuvant therapy should be evaluated for its effect on bacterial colonization factors. The goal of this study was to investigate the effect of *Acacia nilotica* on *H. pylori* activity and colonization factors in order to evaluate its use for the management of *H. pylori* infection.

2. Materials and Methods

Acacia nilotica aerial parts were collected in Birnin Kebbi, Kebbi State, Nigeria. The leaves were identified, authenticated, and stored at the University of Lagos' Department of Botany under the voucher specimen number LUH 7553. The leaves were washed with distilled water and room-dried until crisp at room temperature before being pulverized with a grinder. The plant powder (500g) was soaked in 5 litres of ethanol or distilled water for 24 hours to make the ethanol and aqueous extracts. The ethanol extract was concentrated using a rotary evaporator at 40ºC under reduced pressure, and the aqueous extract was concentrated by freeze-drying.

2.1. Evaluation of the anti-*H. Pylori* Activity

The plant extracts were tested *in vitro* for antimicrobial activity against *H. pylori*, as previously described [9]. Archived *H. pylori* isolated from patients with gastroduodenal illnesses and the strains *p12* and ATCC 700392 were used as control strains. The isolates were suspended to a 3.0 McFarland standard and spread on GC agar containing 8% horse serum, *H. pylori* supplements SR0147 (Oxoid), and vitamin-mix (Vitox; Oxoid). Wells of 7mm diameter were punched on the plates, 30 µL of ethanol and aqueous plant extracts with concentrations ranging from 200 to 3.125 mg/mL were added to each well, and allowed to diffuse at room temperature. The plates were incubated for 48 hours under micro-aerophilic conditions at 37°C. For the remaining assays, only extracts that showed a zone of inhibition at ≤50 mg/mL on the agar dilution assay were considered.

2.2. Determination of Minimum Inhibitory and Minimum Bactericidal Concentration (MIC and MBC)

The MIC and MBC of the plant extract were determined using the methods described previously [10,11]. *H. pylori* log phase cells were suspended in Brucella broth containing 10% foetal bovine serum to a final concentration of 10^8 CFU/mL, and 100 µL of this and 100 µL of the plant extract were added to each well of a microtitre plate to achieve concentrations ranging from 50mg/mL to 0.0195 mg/mL. Clarithromycin (≥95% purity, Sigma-Aldrich, GMBH) was used as a control antibiotic. The absorbance was measured at 630 nm using an automatic ELISA microplate reader, and the plates were incubated at 37ºC for 3 days under micro-aerophilic conditions. Following the incubation period, the plate was agitated and the absorbance was measured again. The two absorbance readings were compared to see if there was any difference in bacterial growth [10] (Bonacorsi et al., 2009), and cell viability was determined by counting the CFU on serum plates after incubation [11].

2.3. Phytochemical Analysis of the Plant Extract

Quantitative determination of the total phenolic, total flavonoid, and total antioxidant capacity of the plant extract was carried out using standard methods. The GC-MS analysis of phytochemical compounds was done using an Agilent Technologies 7890 Gas Chromatography (GC) system equipped with an HP-5 MS capillary column (length: 30m x internal diameter of 0.32mm x film

thickness of 0.25m) and an Agilent Technologies 5975 Mass Spectrometer Detector (MSD). Based on GC retention time, the resulting mass spectra were compared to those of standards in the mass spectrum (Mass Hunter) library.

2.4. Anti-Urease Activity of the Plant Extract

Urease activity was measured using a modified phenol red method (Sgouras et al., 2004) after incubation as described above for determining IC50, 5 µL of the cell suspension was added to 150 µL of urease medium (20% w/v urea and 0.012 % phenol red in phosphate buffer, pH 6.5) in a microtiter plate, and the plate was incubated for 30 minutes at 37°C before measuring absorbance at 630 nm. The reference positive control was hydroxyurea (98% purity; Sigma-Aldrich, UK) at a final concentration of 10 mg/mL.

2.5. Anti-Adhesion Property of the Plant Extract

This was assessed using a previously described method [12]. *H. pylori* cultures in log phase were resuspended in 1 mL of carbonate buffer (0.15 mol/L NaCl/0.1 mol/L Na2CO3, pH 9.0) to a final concentration of 10^8 cfu/mL. Ten microlitres of a 1% fluorescein isothiocyanate (FITC; Sigma, UK) solution in dimethyl sulfoxide (DMSO; Sigma, UK) solution were added to the bacterial suspension and incubated for 1 hour in the dark at room temperature with intermittent mixing. Bacterial cells were recovered by centrifuging at 3,000 xg for 5 minutes, washed thrice with 0.05% PBS-Tween 20 (PBST), and re-suspended in PBS to yield FITC-*H. pylori*.

Gastric tissues free of *H. pylori* and other major pathologic changes were embedded in paraffin and fixed on slides. The slides were deparaffinised in xylene and isopropanol and incubated in a humidified, dark cupboard for 15 minutes with 200 µL of blocking buffer before being washed thrice with PBST. The plant extract was diluted to a final concentration of 0.1 % in blocking buffer (0.2 % BSA, 0.05 % Tween 20, in PBS), and incubated with the FITC-bacteria suspension at room temperature in the dark for 2 hours before centrifugation at 3,000 x g for 5 minutes and washing once with blocking buffer. 3′-Sialyllactose from colostrum, ≥97% (HPLC) (Sigma-Aldrich, UK), was used as the standard.

The FITC-bacteria-plant suspension incubated with the blocked tissue section for 1 hour in a dark, humidified chamber, washed three times with PBST, air-dried in the dark, mounted with a medium containing 4',6-diamidino-2-phenylindole (DAPI) and viewed under a fluorescent microscope. FITC-bacteria suspension were re-suspended in blocking buffer and used as controls. Bacterial adhesion was validated using microscopic observation and a ranking system ranging from (-) for "no bacterial binding" to (+++++) for "very strong bacterial binding."

2.6. In vivo Anti-H. Pylori Activity of the Plant Extracts

Male Swiss albino mice, weighing 14 to 22 g, were kept in sanitized cages with free access to food and water at room temperature and divided into six experimental groups (group 1: Normal control; group 2: ulcerated with Ethanol; group 3: ulcerated with ethanol + *H. pylori* ; group 4: ulcerated with ethanol + *H. pylori* + plant extract (250 mg/kg); group 5: Ulcerated with ethanol + *H. pylori* + plant extract (500 mg/kg); group 6: Ulcerated with ethanol + *H. pylori* + Reference drug (20 mg/kg Clarithromycin); group 7: Ulcerated with ethanol + *H. pylori* + 10 mg/kg Hydroxyurea; and group 8: Ulcerated with ethanol + *H. pylori* + 3′-Sialyllactose (10mg/kg). Ulceration was achieved through intra-gastric inoculation with 0.2 mL/kg ethanol, as described previously [13], and the control groups received the same volume of PBS. Food was restricted for two hours before orogastric infection with *H. pylori*, which lasted seven days, and the plant extracts and standard drugs were administered orally for 14 days, beginning on the third day after ulceration and *H. pylori* infection, as described [14]. The stomachs of the animals were retrieved for further assays after they were sacrificed via cervical dislocation.

2.7. *In Vivo* Estimation of Urease Activity

A portion of the stomach was homogenized in PBS and 5 µL of the homogenate was added to 150 µL of urease medium. Bacterial isolate of *H. pylori* p12 in PBS was used as the positive control, while

sterile PBS was used as the negative control. A colour change from orange to pink denoted the presence of *H. pylori* in the stomach. Colour change was visually observed using a ranking from (-) for "no colour change" to (+++++) for "very strong colour change" based on the intensity of the colour changes observed in the control.

2.8. Fluorescent in Situ Hybridization to Assess in Vivo Bacterial Adhesion

Multiple slides of paraffin-embedded tissue sections from the mice gastric mucosa were prepared and the tissue sections were deparaffinised by immersing the slides for 30 minutes in xylene and ethanol, respectively. The slides were rinsed with water, PBS, and allowed to dry. De-paraffinised slides were air-dried before dipping in fresh sterile absolute ethanol for 3 minutes and allowed to dry again. A falcon tube was prepared with Whatman filter paper saturated with hybridization buffer (0.9M NaCl, 0.02M Tris HCl, pH 8.0; 0.01% SDS, and 30% formamide). Before placing a coverslip over the biopsy tissue, 40 µL of hybridization buffer containing 5 ng/L TRITC-labeled Hpy probe CACACCTGACTGACTATCCCG was added [15]. The slide was hybridized in a hybridization incubator at 46ºC for 1 hour 30 minutes, washed in a pre-warmed wash buffer (0.11M NaCl, 20mM Tris-HCl, pH 8.0; 0.01 % SDS) at 48ºC for 15 minutes, washed again in 1x PBS, and then allowed to dry. The dried biopsy slides were counterstained with 1 g/mL DAPI and incubated in the dark for 5 minutes at room temperature. A fluorescent microscope was used to examine dried slides.

2.9. Statistical Analysis

Statistical analysis was done using the SPSS version 20 software. Results of samples carried out in triplicates were expressed as the mean ± standard deviation (SD). The non-parametric Mann-Whitney U test was used to test the difference in MIC values between the extract and the reference drug, clarithromycin. The non-parametric Kruskal-Wallis test was used to test the difference between the urease levels in the treatment groups in the experimental study. A probability value of $p < 0.05$ was considered statistically significant.

3. RESULTS

3.1. Antimicrobial activity of aqueous *Acacia nilotica* extract

When compared to the ethanol extract, *H. pylori* was more susceptible to the aqueous extract of *A. nilotica*. The *H. pylori* were susceptible to only the aqueous extract at ≤50 mg/mL. The broth microdilution assay showed *Acacia nilotica* aqueous leaf extract had a minimum inhibitory concentration (MIC) of 180 µg/mL, and minimum bactericidal concentration (MBC) of 5 mg/mL against the local strains of *H. pylori* used in this study. The MIC and MBC of the reference clarithromycin were derived as 0.0048g/mL and 0.5g/mL respectively. There was a statistically significant difference in the MIC using the plant extract and Clarithromycin ($p = 0.0001$).

3.2. Phytochemical Constituents of the Extract

The aqueous extract of *A. nilotica* showed highly detectable levels of phenolic compounds, cardiac glycosides, tannins, saponins and steroids; moderately detectable levels of terpenoids, and detectable levels of flavonoids, quinones and alkaloids. The quantitative analysis of the aqueous *A. nilotica* extract revealed that it contained a high concentration of phenols (71.4 mg/100g), cardiac glycosides (84 mg/100g), tannins (128.6 mg/100g), saponins (80.4 mg/100g), and alkaloid constituents (33.09 mg/100g) (Fig. 1A). Evaluation of the total phenolic, total flavonoids, and total antioxidant capacity of the plant extracts is presented in Fig. 1B. The result of the analysis on aqueous *Acacia nilotica* extract showed a high total phenolic content of 154 mg gallic acid equivalent per gram of extract (mg GAE/g), a total flavonoid activity of 8 quercetin equivalent (QE)/gram extract, and a high antioxidant activity of 312 mg ascorbic acid equivalent/g extract (Fig. 1B).

Identification of the constituents based on the chromatogram from the gas chromatography–mass spectrometry (GC-MS) of the aqueous extract was done based on the peak area, retention time, and molecular formula, which revealed the presence of 75 bioactive compounds, of which 13 had no match in the library used (Fig. 2). The major peaks 21, 22, 37 and 39 with retention time 9.22, 9.592, 13.949, 14.497 had a percentage composition of 28.10, 6.05, 9.42, and 14.00 with the structure

prediction indicating one aromatic ring compound (2-Cyclohexen-1-one-4,4-d2, 6,6-dimethyl-), 4,4-dimethyl-2-(3-phenyl-2-thienyl 2-oxazoline, an aliphatic chain compound (-2-methyl(3-D) propenoic acid) and a cyclic compound 2,5,5-trimethyl-1-cyclopent-2-enon) respectively. Additionally peak 18 with retention time 8.269 had a percentage area of 0.43 with structure predicted to be methylbenzaldehyde (2-methoxy-6-methyl-benzaldehyde).

3.3. Anti-Urease Activity of the Extract

The *in vitro* anti-urease effect of aqueous extract of *Acacia nilotica* leaf, characterized by the percentage inhibition curve for urease, is presented in Fig. 3. The percentage urease activity of *H. pylori* steadily dropped with the introduction of the plant extract. Urease inhibition in *H. pylori* was at 92% inhibition at 10 mg/mL of the plant extract, and urease was completely inhibited (100% inhibition) at 25 mg/mL. With 10 mg/mL of the reference anti-urease agent, hydroxyurea, urease activity was completely inhibited. The results for the *in vivo* effect of *Acacia nilotica* on urease activity are presented in Table 1. There was a statistically significant difference in the urease levels of the treatment group and the untreated groups ($p < 0.001$), but there was no statistically significant difference in the urease levels of the group treated with 250mg and 500mg of the plant extract and the group treated with the reference drug, clarithromycin ($p = 0.53$) and when compared with 10 mg/mL hydroxyurea ($p = 0.53$).

Table1. *Effect of Acacia nilotica Leaf Extract on Urease Activity of H. pylori infected Mice*

Experimental Group	Urease production
Negative control (PBS)	-
Positive Control (*H. pylori* isolate *p12*)	+++++
Group 1	+
Group 2	++
Group3	+++
Group 4	++
Group 5	++
Group 6	++
Group 7	++
Group 8	++

Key: - = not detected; + = less detected; ++/+++ = moderately detected; ++++ = Highly detected; +++++ = Very highly detected

3.4. Anti-Adhesion Property of the Extract

The fluorescent microscopy of the *in situ* hybridization assay carried out to evaluate the anti-adhesion property of aqueous extract of *Acacia nilotica* is shown in Fig. 4. A very strong bacterial binding was noticed on all triplicate fluorescent microscopy slides for control tissues incubated with FITC-bacteria suspension re-suspended in blocking buffer. However, no bacterial binding was seen in tissue samples incubated with the plant extract and the reference anti-adhesion agent, 3-Sialyllactose. *Acacia nilotica* exhibited high anti-adhesion properties with no bacteria adhesion seen on the stomach lining of the tissue samples. The result of the experiment on the *in vivo* adhesion of *H. pylori* to mouse gastric tissues is presented in Fig. 5. In the groups treated with 250mg/kg *Acacia nilotica* aqueous leaf extract and 10mg/kg 3′-Sialyllactose, fluorescent *in situ* hybridization revealed no/scarce bacterial adhesion. However, no bacterial adhesion was seen in the group treated with 500 mg/kg of the *Acacia nilotica* aqueous leaf extract, but a high bacterial binding was seen in the untreated group.

4. DISCUSSION

In this study, we used a combination of *in vitro*, *in situ*, and *in vivo* assays to determine the anti-*H. pylori* activity and effects on *H. pylori* colonization factors of *A. nilotica*, a plant indigenous primarily in Northern Nigeria and commonly used for gastrointestinal disorders. Preliminary testing of aqueous and ethanolic extracts of *A. nilotica* revealed that the aqueous extract inhibited *H. pylori* better than the ethanolic extract. This finding contradicted previous research that found organic extracts of medicinal plants to have higher anti-*H. pylori* activity than aqueous extracts [16][16] . In a similar study, methanol and acetone extracts of *A. nilotica* were found to be more effective against *H. pylori* than aqueous extract [17].

Acacia nilotica aqueous leaf extract had MIC of 180 µg/mL and MBC of 5 mg/mL against *H. pylori*. The MIC of medicinal plants can be divided into three categories: strong (MIC: <10 µg/mL), strong-moderate (MIC: 10 - 100 µg/mL); weak-moderate (MIC: 100 - 1000 µg/mL); and weak (MIC: >1000 µg/mL) [18]. The crude extract of aqueous *A. nilotica* leaf extract exhibited weak-moderate activity against *H. pylori*, with a MIC of 180 µg/mL according to this classification. This is consistent with the findings of another study, which found a MIC value of 128 µg/mL for *A. nilotica* methanol extract against *H. pylori* (Amin et al., 2013). The majority of studies examining medicinal plants' anti-*H. pylori* activity reported weak-moderate activity, with only a few plants exhibiting high-moderate activity [18].

The presence of phytochemicals like phenols, flavonoids, alkaloids, terpenes, and steroids has been shown to exhibit bioactive and antimicrobial activity [19,20]. The presence of moderate to copious amounts of these phytochemicals in *A. nilotica* may be responsible for its observed moderate anti-microbial properties against *H. pylori*. Phenolic compounds are usually distributed in plants as phenolics, simple phenols, and phenols, while tannins are also an example of a polymeric phenolic substance. Tannins have been reported as one of the most vital constituents with anti-ulcer and gastro-protective activities [19]. Purified forms of phenolic compounds such as tannins have also been shown to have anti-*H. pylori* activity [21].

Bacterial colonization may be significantly inhibited by anti-adhesion phytochemical agents, which block or treat bacterial infection. The presence of phytochemicals like fatty acids, phenols, flavonoids, alkaloids, terpenes, and steroids has been shown to exhibit bioactive and antimicrobial activity [19,20,22]. The GCMS analysis of the plant extracts showed a high percentage composition of the phenolic compounds 2-Cyclohexen-1-one-4,4-d2, 6,6-dimethyl- and 2,5,5-trimethyl-1-cyclopent-2-enon, and the fatty acid, -2-methyl (3-D) propenoic acid. Though seen in a moderate percentage, the compound, 4,4-dimethyl-2-(3-phenyl-2-thienyl 2-oxazoline, is a Polyoxazoline. Polyoxazolines and their aggregates have been reported to enhance antimicrobial action in some bacteria [23]. The presence of this moderate Polyoxazoline may be responsible for the minimal antibacterial activity seen by *A.nilotica* plant extract on *H. pylori*. The GCMS analysis also showed the presence of a low percentage composition of the phenolic derivative, methyl benzaldehyde, whose derivative has been reported to inhibit biofilm formation at lower concentrations when compared to a higher concentration [24].

Urease production helps *H. pylori* survive the harsh acidic conditions of the stomach. The ability of a plant extract to inhibit urease production in *H. pylori* indicates its ability to prevent *H. pylori* colonization of the stomach. Though this ability does not kill *H. pylori*, it makes the bacteria susceptible to the harsh acidic stomach conditions. The aqueous extract of *A. nilotica* was able to inhibit urease production in *H. pylori*, and showed urease inhibition in a dose-dependent relationship *in vitro*. This may be associated with the inhibition of the urease enzyme in the bacteria. This high urease inhibition activity by *A. nilotica* has also been reported in another study [17]. The anti-urease activity of *A. nilotica* observed was similar to that of Hydroxyurea and clarithromycin. However, it is important to note that clarithromycin has a bactericidal and anti-bacterial effect on *H. pylori*. Hence, the lack of activity seen with the use of clarithromycin could be as a result of its bactericidal effect and not its anti-urease effect.

Another colonization factor of *H. pylori* is its adhesion to the host receptors using adhesins. This study showed that *Acacia nilotica* had an anti-adhesion property, which was observed using both *in situ* and *in vivo* experiments. In the *in situ* experiment, the aqueous crude extract of *Acacia nilotica* was able to prevent bacteria binding to gastric tissues. In the *in vivo* experiments, the stomach lining of the experimental groups treated with *Acacia nilotica* showed little or no bacteria binding. The anti-adhesion property exhibited by the plant suggests that it is able to prevent *H. pylori* colonization of the stomach by preventing it from adhering to the host cell to acquire the nutrients necessary for replication [25]. Though studies have evaluated the anti-microbial activity of *Acacia nilotica*, to the best of our knowledge, there are currently no studies that have assessed the anti-adhesion ability of *Acacia nilotica* against *H. pylori*. Though the antimicrobial activity of the plant is moderate on *H. pylori*, the ability of the plant extract to affect the colonization factors of *H. pylori in vivo* signifies that using *Acacia nilotica* will make *H. pylori* susceptible to the host defence system, which may eventually result in the eradication of the bacteria in the stomach.

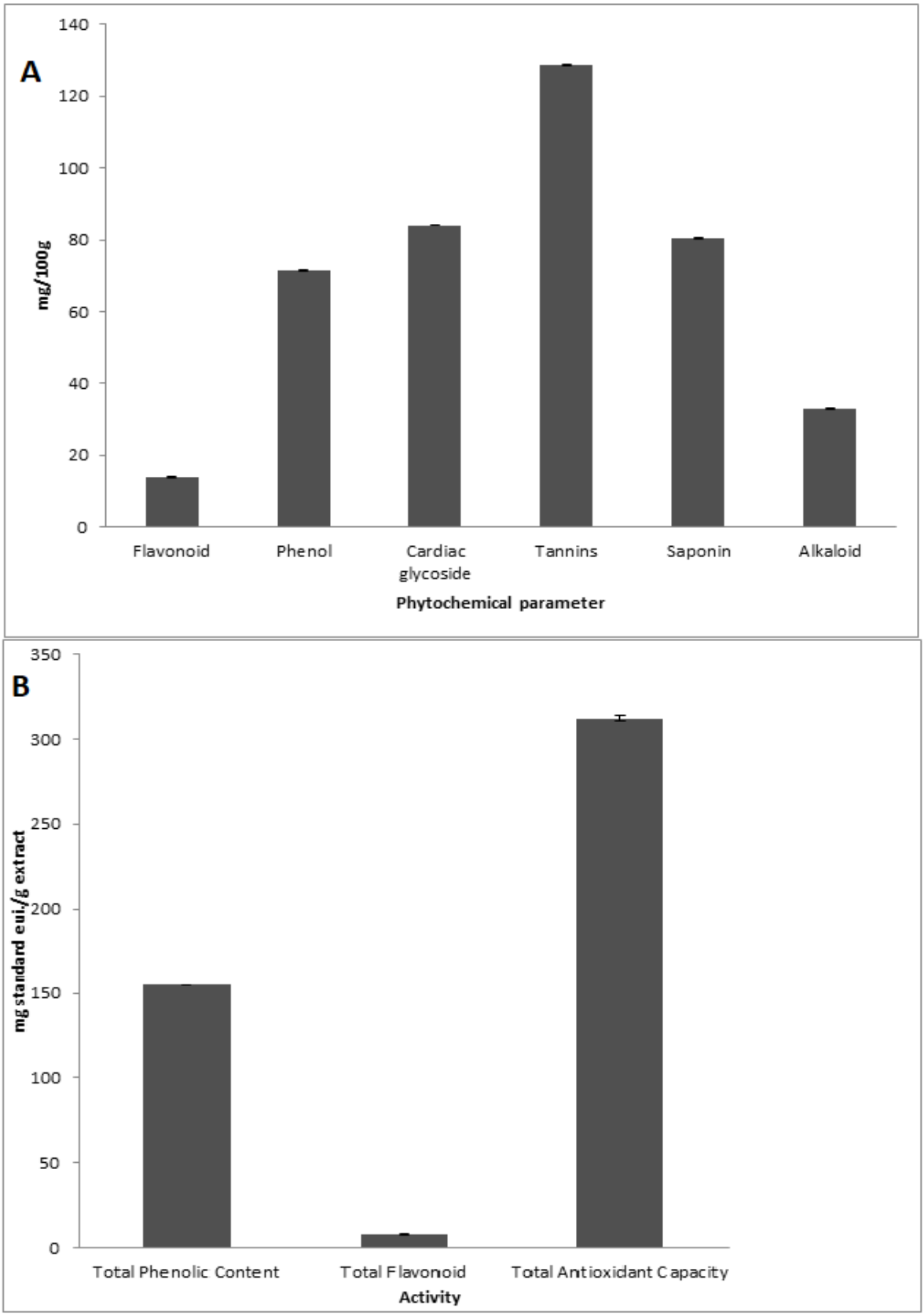

Fig1. ***Phytochemical constituents of the aqueous Acacia nilotica extract. A. – Quantitative Phytochemical components; B - Total phenolic, flavonoid, antioxidant capacity of the extract.*** *Analyses were done in triplicates and values are expressed as Mean ± SEM. Total phenolic content is expressed as mg gallic acid equivalent/g extract; total flavonoid is expressed as mg quercertin equivalent/g extract; total antioxidant capacity is expressed as mg ascorbic acid equivalent/g extract.*

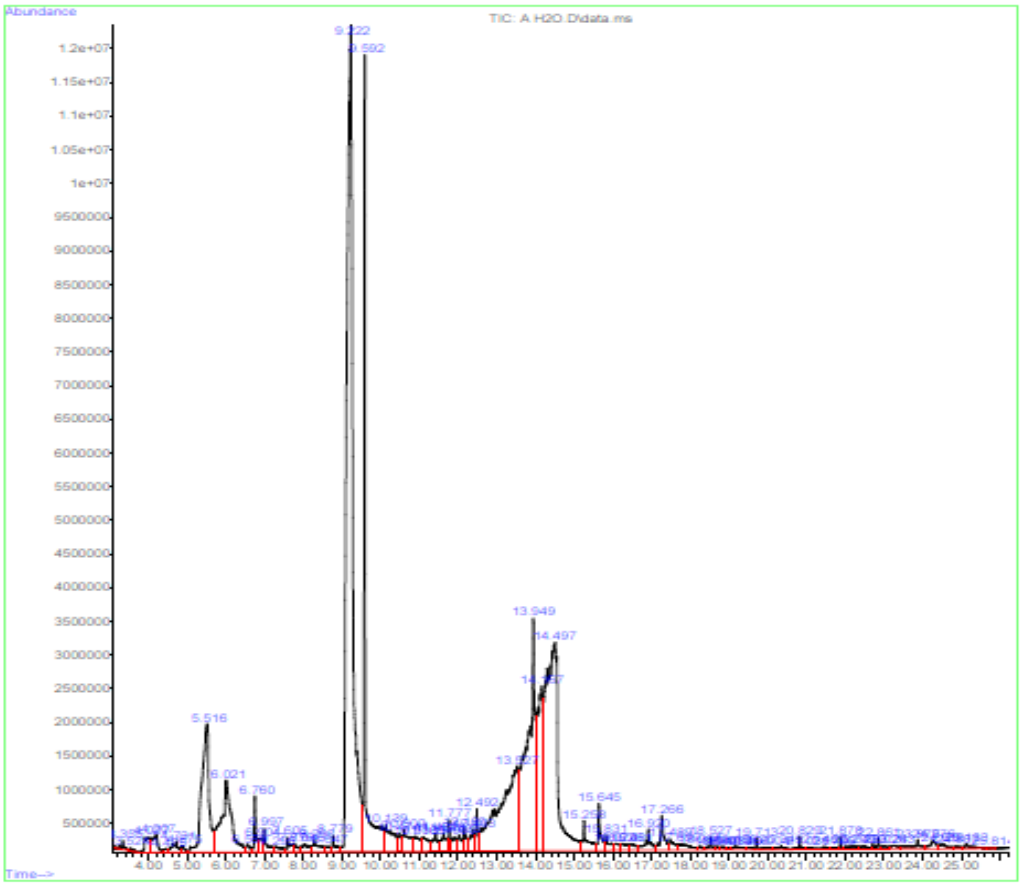

Fig2. *GC-MS chromatogram of aqueous Acacia nilotica extract.*

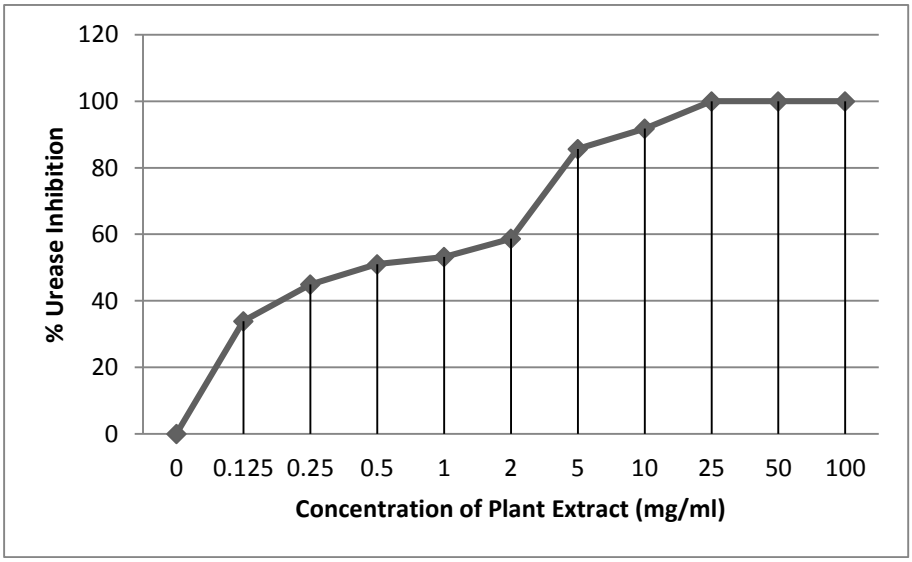

Fig3. *In vitro effect of aqueous Acacia nilotica extract on urease activity*

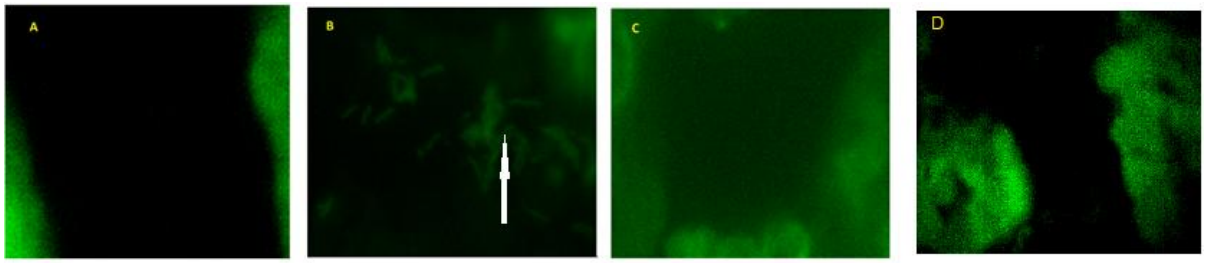

Fig4. *Fluorescent in situ hybridization films for the in vitro adhesion study*

a = Stomach lining with no bacteria; b = Stomach lining with non-treated bacteria (arrow shows the presence of bacteria binding); c = Stomach lining with bacteria treated with aqueous A. nilotica leaf extract (no bacteria binding); d = Stomach lining with bacteria treated with 1mg/ml 3′-Sialyllactose (no bacteria binding).

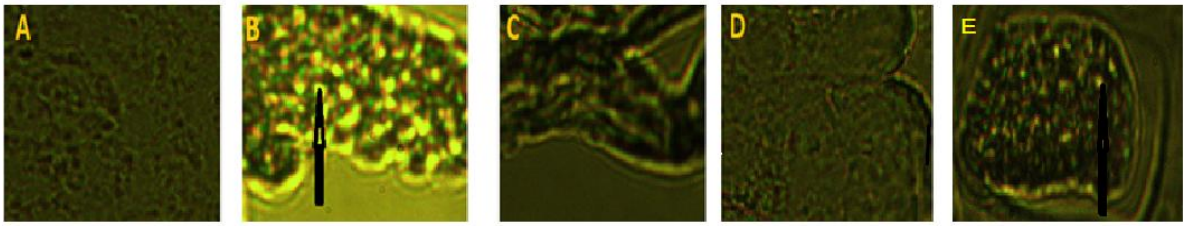

Fig5. *In vivo adhesion of H. pylori to mice stomach tissues*

a = Stomach lining of the normal control; b = Stomach lining of the group Ulcerated with ethanol + *H. pylori*; c = Stomach lining of the group ulcerated with ethanol + *H. pylori* + plant extract (250 mg/kg); d = Stomach lining of the group Ulcerated with ethanol + *H. pylori* + plant extract (500 mg/kg); e = Stomach lining of the group Ulcerated with ethanol + *H. pylori* + 3′-Sialyllactose (10mg/kg).

5. Conclusion

In conclusion, our study indicated that *Acacia nilotica* showed moderate antimicrobial activity against *H. pylori*, high anti-urease activity, and high anti-adhesion property. Therefore, the aqueous extracts of this plant, which is already in use for the treatment of gastroduodenal disorders in the Northern part of Nigeria, may be explored for its pharmaceutical application for the possible treatment of *H. pylori* infection and gastric ulcers. Though the crude plant extract was used in this study, further studies are underway to identify the main bioactive principles responsible for the different activities possessed by the plant.

Ethics Statement

The study was approved by the Institutional Review Board of the Nigerian Institute of Medical Research. Animal care and use were conducted in compliance with the principles of the European Community standards for laboratory animal use and care (EEC Directive 86/609/EEC).

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