Anti-Helicobacter pylori Activity of Abelmoschus esculentus L. Moench (okra): An in vitro Study

Taiye A Olorunnipa1, Christopher C Igbokwe1, Temitope O Lawal1, Bolanle A Adeniyi2* and Gail B. Mahady2

1Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan, Nigeria
2Department of Pharmacy Practice, University of Illinois Chicago, USA

Abstract

The anti-Helicobacter pylori activity of the methanol and hexane extracts of Abelmoschus esculentus L. Moench (okra) dried fruits were evaluated on forty-one clinical isolates and a standard ATCC 43504 strain by the use of agar well diffusion technique. The methanol extract of A. esculentus showed A. esculentus L. Moench (okra) dried fruit had inhibitory effects against Helicobacter strains; with diameters zone of inhibition between 13 and 28 mm on 32 out of the 42 isolates tested. No noticeable zone of inhibition was observed from the hexane extract of the tested plant on all the H. pylori strains tested. The bioactive methanol extract of A. esculentus demonstrated A. esculentus L. Moench (okra) dried fruit had Minimum Inhibitory Concentration (MIC) values of 70 to 85 mg mL⁻¹ on selected susceptible strains except H. pylori AT CC 43504 which had MIC value of 250 mg mL⁻¹. The time-kill study of the methanol extract of A. esculentus on H. pylori BAA009, H. pylori BAA026 and H. pylori ATCC 43504, revealed a decline in the surviving population of the organisms after 8 h of exposure to the methanol extracts of A. esculentus L. Moench dried fruit at doses equivalent to MIC × MIC and 4 × MIC, and a total kill of the population at 24 h.

Materials and Methods

Plant collection, extraction, and preparation of extracts

Dried fruits of A. esculentus L. Moench (okra) were purchased from Bodija Market, Ibadan, Oyo State, Nigeria; between the months of December 2010 and March 2011; and then identified and authenticated at the Department of Botany and Microbiology, University of Ibadan, and Forest Research Institute of Nigeria (FRIN), Ibadan, Oyo State. Voucher specimen was deposited at FRIN with herbarium number FHI 109558. The fruits were dusted and air dried at room temperature for 4 to 5 weeks and then grounded to coarse powder using a dry electric mill (Moulinex). The pulverized plant material (8.6 kg) was extracted (in smaller portions) by subjecting it to exhaustive Soxhlet extraction with n-hexane and methanol in

Keywords: Anti-Helicobacter pylori; Abelmoschus esculentus; Fruit; Kill kinetics; Methanol; Hexane

Introduction

Helicobacter pylori is a Gram-negative spiral-shaped, fastidious, microaerophilic bacillus [1] hun an pathogen currently being investigated worldwide due to its prevalence in almost 50% of the world's population and has been implicated as a major etiologic agent of chronic gastritis, peptic ulcer disease (PUD), gastric adenocarcinoma, and lymphoma [2,3]. Since its first acceptance by the international guidelines in 1996, the standard first-line treatment options for H. pylori eradication involves triple therapies which utilize an antisecretory agent (usually a Proton-Pump Inhibitor (PPI)) and two antimicrobial agents most of the ten selected from amoxicillin, clarithromycin, and metronidazole [3]. In the last decade however, a progressive decline in cure rates below the acceptable level of 80% has been reported [4] with increasing antimicrobial resistance of H. pylori in many countries leading to difficulty in the successful treatment of H. pylori infections [5,6]. Estimates suggest that ~80% of people living in developing countries depend primarily on traditional medicine [7] with the use of herbs from plants as major source for treating diseases [8]. One of such common plant readily available in developing countries like Nigeria is Abelmoschus esculentus L. Moench. Also known as lady's finger or okra, A. esculentus is edible and well known for its nutritional value and healing properties such as anticancer, reduced heart attack, lower blood cholesterol, relieve intestinal disorder, relieve inflammation of the colon, relieve diverticulitis, relieve stomach ulcer, neutralize acid, lubricate large intestine, treatment of lung inflammation, treat ent of irritable bowel, keep joints limber, as well as the treat ent of sore throats, burns, reducing poisonings and psoriasis [9-12]. A. esculentus has also been shown to possess antibacterial properties against infectious disease causing bacterial pathogens such as Bacillus subtilis, Streptococcus pyogens, Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli, Proteus mirabilis and Pseudomonas aeruginosa [13], Rhodococcus opacus, Mycobacterium sp. and M. aurum, Staphylococcus aureus, Escherichia coli, and Xanthobacter py2 [14], inhibit the adhesion of Helicobacter pylori to human gastric mucosa [15] and inhibits the adhesion of Campylobacter jejuni to mucosa isolated from porcine in vivo but not in vivo [16]. In Nigeria and most developing countries, H. pylori infection is a public-health issue [17]. The aim of this study is to evaluate the in vitro anti-Helicobacter pylori activity of A. esculentus specifically to determine its zone of inhibition, Minimum Inhibitory Concentration (MIC) and kill rate with time on the organism.

Materials and Methods

Plant collection, extraction, and preparation of extracts

Dried fruits of A. esculentus L. Moench (okra) were purchased from Bodija Market, Ibadan, Oyo State, Nigeria; between the months of December 2010 and March 2011; and then identified and authenticated at the Department of Botany and Microbiology, University of Ibadan, and Forest Research Institute of Nigeria (FRIN), Ibadan, Oyo State. Voucher specimen was deposited at FRIN with herbarium number FHI 109558. The fruits were dusted and air dried at room temperature for 4 to 5 weeks and then grounded to coarse powder using a dry electric mill (Moulinex). The pulverized plant material (8.6 kg) was extracted (in smaller portions) by subjecting it to exhaustive Soxhlet extraction with n-hexane and methanol in
succession. Extracts were collected, dried under reduced pressure, weighed, and stored at −20°C for 24 h before use. Stock solutions of lyophilized extracts were reconstituted in 20% DMSO with final concentrations of 100 to 400 mg/ml prepared for the initial screening. Lower concentrations in the range 20 to 300 mg/ml were also prepared to determine the Minimum Inhibitory Concentrations (MICs) of the bioactive crude extracts.

**Antimicrobial agents**

The chemotherapeutic agents used in the test as positive control were Gentamicin 100 μg/mL (Nicholas Laboratories Limited, England), Ofloxacin in 100 μg/mL and Metronidazole 100 μg/mL, while the negative control was 20% DMSO.

**Phytochemical screening**

Phytochemical screening was carried out to detect the presence of secondary metabolites such as anthraquinones, tannins, saponins, alkaloids, and carbenolides using methods described by Harborne [18].

**Strains of *Helicobacter pylori* and culture methods**

Forty-one clinical isolates and a standard strain ATCC 43504 were used for this investigation. All the clinical isolates were isolated, characterized, and identified at the Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan, Nigeria; while the ATCC strain was from College of Pharmacy, University of Illinois, Chicago, USA.

**Susceptibility testing**

Susceptibility was determined using the agar well diffusion technique. A 0.1 ml aliquot of logarithmic phase broth culture of each bacterium (optical density equivalent to 10^7-10^8 cfu/ml) was used to seed sterile molten Mueller-Hinton agar (OXOID) medium with 5% sterile horse blood maintained at 45°C. The seeded plates were allowed to dry in the incubator at 37°C for 20 min. A standard cork borer (8 mm diameter) was used to cut uniform wells on the surface of the agar, into which was added increasing concentrations of the test extract dissolved in 20% DMSO. A pre-incubation diffusion of the extracts into the seeded medium was allowed for 1 h. Plates were incubated at 37°C in an automatic CO₂-O₂ incubator under microaerophilic conditions (85% N₂, 10% CO₂ and 5% O₂) for 2-3 days after which diameters of zones of inhibition (mm) were measured. Since each of the extracts was reconstituted in 20% DMSO, these diluents were included in each plate as a solvent control besides the chemotherapeutic agents included as positive controls. This method has been adopted from previous published procedures [19].

**Determination of minimum inhibitory concentrations**

Minimum Inhibitory Concentrations (MICs) were performed by a modification of standard agar dilution method procedures as previously described [20]. Extracts were tested at various concentrations. The positive control antibiotic included was ofloxacin. The MICs were determined after 3 to 5 days of incubation at 37°C under microaerophilic conditions. The MIC was regarded as the lowest concentration that showed no visible growth from a duplicate experiment.

| Name Of Extract Plant/Solvent | Alkaloids | Saponins | Cardenolides | Anthraquinones | Tannins | Flavonoid |
|------------------------------|-----------|----------|--------------|----------------|---------|----------|
| A. esulentus (L.) Hexane     | +         | +        | -            | -              | +       | N.D      |
| A. esulentus (L.) Methanol   | +++       | +        | ++           | -              | +       | N.D      |

Note: +=Low (trace amount), +++=Medium concentration, +++=High concentration, N.D=Not done

**Time-kill Assay**

**Determination of bactericidal activity of the methanol extract of *A. esulentus***

The viable counting technique was employed for this assay as previously described [21]. An overnight broth culture in 4.5 ml of Tryptic soy broth inoculated in a static growth condition of each organism was made. Two of the *H. pylori* strains coded BAA009 and *H. pylori* BAA026 and a standard strain ATCC 43504 were used for this experiment. A 0.5 ml of each culture was subculture into a warm (37°C) 4.5 ml Tryptic Soy broth and incubated for 90 min using a Gallenkamp orbital incubator to give a logarithmic phase culture. A 0.1 ml of the logarithmic phase culture was then inoculated into a warm 4.9 ml of Tryptic Soy broth containing the test extract to give a dilution of the culture (equivalent to approximately 1 × 10⁸ colony forming units) and the required concentration of the extract. A loopful of the test sample (extract-culture mixture) was withdrawn immediately, diluted out in Tryptic Soy broth and 0.2 ml of 1:1000 dilution plated on an oven dried Mueller-Hinton agar to give control time 0 min count. Samples were taken at 30 min, 1, 2, 4, 6 and 24 h. The procedure was carried out in duplicate. Plates were incubated at 37°C for 24 h before counting the colonies. Control plates for negative and positive controls were also incubated. The number of colony forming unit were counted after the period of incubation. The numbers of surviving bacterial cells per ml were calculated by taking into consideration the dilution factor and the volume of the inoculum. All the procedure was repeated for 2 × MIC and 4 × MIC. A graph of percentage viable count against time in hour (h) was plotted to show the rate of kill of the test organisms after duplicate experiments.

**Results**

Bactericidal effects against *Helicobacter strains*; with diameters zone of inhibition of the ex tract between 11 and 28 mm, in 31 out of the 42 isolates tested. No noticeable zone of inhibition was observed by the hexane extract of the tested plant on all the *Helicobacter strains* tested.

The Phytochemical screening of the methanol and hexane extracts of *A. esulentus* (data shown in Table 1) showed the presence of alkaloids, saponins, cardenolides, anthraquinones and tannins. These various plant metabolites have earlier been reported to possess medicinal, antimicrobial and physiological activities [22,23]. Many phytomedicines exert their effects through the additive or synergistic action of several compounds acting at a single or multiple target sites associated with physiological process [25]. It is noteworthy to state that a large concentration of alkaloids were observed in this study, with all the fractions obtained from the methyl extract possessing different degrees of antimicrobial activities on *H. pylori* strains.

The MICs of methanol extract of *A. esulentus* on the entire test *H. pylori strains* in Table 1 were observed to be generally high. This is similar to previous works on crude extracts of plants by other researchers, who reported high MIC values against their test microorganisms [26-28]. However, the MIC values confirmed the presence of antibacterial effects of *A. esulentus* dried fruit with MIC

---

Citation: Olorunnipa TA, Igbokwe CC, Lawal TO, Adeniyi BA, Mahady GB (2013) Anti-*Helicobacter pylori* activity of *Abelmoschus esulentus* L. Moench (okra): An in vitro study. Clin Microbial 2: 132. doi: 10.4172/2327-5073.1000132

Table 1: Phytochemical Analysis of the Crude Extracts of *A. esulentus*. 
values of 70 to 85 mg/mL for both extracts on selected susceptible strains except *H. pylori* ATCC 43504 which had MIC of 14 mm and above ranged between 70 to 85 mg/mL. The time-kill study of the methanol extract of the plant on *H. pylori* BAA009, *H. pylori* BAA026 and *H. pylori* ATCC 43504 are shown in Figures 1-3.

**Discussion**

In this study, the anti-*H. pylori* activity of the methanol and hexane extracts of *A. esulentus* dried fruits was evaluated. The antimicrobial screening results of the anti-*Helicobacter* activity of the extracts by the use of agar well diffusion technique were presented in Table 2. The MICs of 13 out of the 42 isolates of *H. pylori* using methanol extracts of *A. esulentus* was determined, while two of the *H. pylori* strains coded BAA009 and *H. pylori* BAA026 and a standard strain ATCC 43504 were used for bactericidal (kill) studies. The studies showed that the methanol extracts of *A. esulentus* dried fruit had similar MIC (>512 µg/mL) result of anti-*H. pylori* activity of while *H. pylori* Methanol extract (mg/ml) Hexane extract (mg/ml) MIC (mg/ml) Ofloxacin (µg/ml) Gentamicin (µg/ml) Metronidazole (µg/ml) 20% DMSO

| *H. pylori* | Methanol extract (mg/ml) | Hexane extract (mg/ml) | MIC (mg/ml) | Ofloxacin (µg/ml) | Gentamicin (µg/ml) | Metronidazole (µg/ml) | 20% DMSO |
|-------------|--------------------------|------------------------|-------------|-------------------|-------------------|----------------------|----------|
| BAA003      | -                        | -                      | N.E         | 20 ± 0.0          | 23 ± 0.0          | 4                  | 0        |
| BAA003      | -                        | -                      | N.E         | 22 ± 0.0          | -                 | 0                   | 0        |
| BAA003      | -                        | -                      | N.E         | 22 ± 0.0          | -                 | 0                   | 0        |
| BAA003      | 16 ± 0.0                 | N.E                    | -           | 85                | 26 ± 0.5          | 26 ± 0.5            | 0        |
| BAA003      | 17 ± 0.0                 | N.E                    | -           | 80                | 26 ± 0.0          | 22 ± 0.5            | 0        |
| BAA003      | -                        | -                      | N.E         | -                 | 24 ± 0.5          | 25 ± 0.5            | 0        |
| BAA003      | -                        | -                      | N.E         | -                 | 18 ± 0.5          | 20 ± 0.5            | 0        |
| BAA003      | -                        | -                      | N.E         | -                 | 22 ± 0.0          | 16 ± 0.0            | 0        |
| BAA003      | -                        | -                      | N.E         | -                 | 24 ± 0.5          | 22 ± 0.0            | 0        |
| BAA003      | -                        | -                      | N.E         | -                 | 22 ± 0.5          | 26 ± 0.0            | 0        |
| BAA003      | -                        | -                      | N.E         | -                 | 22 ± 0.0          | 26 ± 0.0            | 0        |
| BAA003      | -                        | -                      | N.E         | -                 | 24 ± 0.0          | 22 ± 0.5            | 0        |
| BAA003      | -                        | -                      | N.E         | -                 | 22 ± 0.0          | 26 ± 0.5            | 0        |
| BAA003      | 17 ± 0.5                 | N.E                    | -           | 85                | 26 ± 0.0          | 22 ± 0.5            | 0        |
| BAA003      | -                        | -                      | N.E         | -                 | 22 ± 0.0          | 26 ± 0.0            | 0        |
| BAA003      | -                        | -                      | N.E         | -                 | 22 ± 0.5          | 26 ± 0.0            | 0        |
| BAA003      | 15 ± 0.0                 | N.E                    | -           | 80                | 22 ± 0.0          | 22 ± 0.5            | 0        |
| BAA003      | 16 ± 0.0                 | N.E                    | -           | 80                | 30 ± 0.5          | 26 ± 0.0            | 0        |
| BAA003      | 16 ± 0.5                 | N.E                    | -           | 80                | 22 ± 0.0          | 22 ± 0.5            | 0        |
| BAA003      | 13 ± 0.5                 | N.E                    | -           | 80                | 22 ± 0.0          | 22 ± 0.5            | 0        |
| BAA003      | 17 ± 0.0                 | N.E                    | -           | 85                | 20 ± 0.0          | 20 ± 0.0            | 0        |
| BAA003      | -                        | -                      | N.E         | -                 | 22 ± 0.0          | 26 ± 0.5            | 0        |
| BAA003      | 21 ± 0.5                 | -                      | -           | 85                | 22 ± 0.0          | 20 ± 0.0            | 0        |
| BAA003      | 17 ± 0.0                 | -                      | -           | 85                | 22 ± 0.5          | 20 ± 0.0            | 0        |
| BAA003      | 19 ± 0.5                 | N.E                    | -           | 80                | 22 ± 0.0          | 16 ± 0.5            | 0        |
| BAA003      | 28 ± 0.5                 | N.E                    | -           | 80                | 18 ± 0.0          | 23 ± 0.0            | 0        |
| BAA003      | 18 ± 0.0                 | N.E                    | -           | 80                | 22 ± 0.0          | 22 ± 0.5            | 0        |
| BAA003      | 23 ± 0.0                 | N.E                    | -           | 80                | 22 ± 0.0          | 22 ± 0.5            | 0        |
| BAA003      | 15 ± 0.0                 | N.E                    | -           | 80                | 22 ± 0.0          | 22 ± 0.5            | 0        |
| BAA003      | 19 ± 0.0                 | N.E                    | -           | 80                | 22 ± 0.0          | 22 ± 0.5            | 0        |
| BAA003      | 17 ± 0.0                 | N.E                    | -           | 80                | 22 ± 0.0          | 22 ± 0.5            | 0        |
| BAA003      | 20 ± 0.5                 | N.E                    | -           | 80                | 22 ± 0.0          | 22 ± 0.5            | 0        |
| BAA003      | 16 ± 0.0                 | N.E                    | -           | 80                | 24 ± 0.0          | 24 ± 0.5            | 0        |
| BAA003      | 18 ± 0.0                 | N.E                    | -           | 80                | 24 ± 0.0          | 24 ± 0.5            | 0        |
| BAA003      | 22 ± 0.5                 | N.E                    | -           | 80                | 34 ± 0.0          | 26 ± 0.0            | 0        |
| BAA003      | 19 ± 0.0                 | N.E                    | -           | 80                | 34 ± 0.0          | 26 ± 0.0            | 0        |
| BAA003      | 21 ± 0.0                 | N.E                    | -           | 80                | 37 ± 0.0          | 24 ± 0.0            | 0        |
| BAA003      | 22 ± 0.0                 | N.E                    | -           | 80                | 22 ± 0.0          | 22 ± 0.0            | 0        |
| BAA003      | 17 ± 0.5                 | N.E                    | -           | 80                | 22 ± 0.0          | 22 ± 0.0            | 0        |
| BAA003      | 17 ± 0.0                 | N.E                    | -           | 80                | 22 ± 0.0          | 22 ± 0.0            | 0        |
| BAA003      | 22 ± 0.0                 | N.E                    | -           | 80                | 22 ± 0.0          | 22 ± 0.0            | 0        |
| BAA003      | 21 ± 0.0                 | N.E                    | -           | 80                | 22 ± 0.0          | 22 ± 0.0            | 0        |
| BAA003      | 23 ± 0.5                 | N.E                    | -           | 80                | 22 ± 0.0          | 22 ± 0.0            | 0        |
| BAA003      | 20 ± 0.0                 | N.E                    | -           | 80                | 22 ± 0.0          | 22 ± 0.0            | 0        |
| ATCC43504   | -                        | 26 ± 0.0               | -           | 250               | 21 ± 0.0          | 15 ± 0.0            | 0        |

*Table 2: Antimicrobial susceptibility of Helicobacter pylori to methanol extracts of A. esulentus. Diameter of zones of inhibition (mm) and MICs.*

*Result is average of duplicate experiment. -=No activity, N.E=Not evaluated. Diameter of cork borer=8 mm. Note: The MICs of Ofloxacin=40 µg/ml, Gentamicin=80 µg/ml, Metronidazole=N.E on all the *H. pylori* strains
investigating the anti-H. pylori and anti-internalization activities of thirteen Thai plant extracts used for gastric ailments in traditional medicine. The time-kill study of the m ethanol extracts on H. pylori BAA009, H. pylori BAA026 and H. pylori ATCC 43504 as shown in Figures 1-3, revealed a dose dependent decline in population after 8 h of exposure to the m ethanol extracts at doses equivalent to MIC, 2 × MIC and 4 × MIC, followed by a total kill of the population at 24 h. A higher kill rate by the extract at higher concentration (4 × MIC) was generally observed, suggesting resistance of the H. pylori strains to lower concentrations. The bactericidal activity was observed to be dependent on time and dose/concentration as the percentage reduction in viable count of surviving population increased with increase in exposure time and concentration of the extracts. This is similar to previous kinetics study [30].

H. pylori infection is associated with chronic gastritis, gastric and duodenal ulcers and gastric cancer in humans [31]. Several treatment regimens have been developed and proved to eradicate H. pylori with a cure rate of up to 90% [32]. However, these regimens may have side effects, poor compliance, and antibiotic resistance [33]. Therefore, alternative antimicrobial agents such as A. esculentus L. Moench with fewer side effects are necessary for the treatment of H. pylori infection in developing countries, especially as they are edible and readily available.

Conclusion

The anti-H. pylori activities exhibited by A. esculentus L. Moench suggests its local use in the treatment of gastro-intestinal diseases associated with the H. pylori species. Our result show the MIC value does not show potent activity to focus on isolation. However, isolation for phytochemical characterization of active components can be done. Moreover, since this plant is edible it can be safely taken in copious amounts regularly. Thus, it is a potential health food source.

References

1. Marshall BJ, Warren JR (1984) Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1: 1311-1315.

2. (1994) Infection with Helicobacter pylori. IARC Monogr Eval Carcinog Risks Hum 61: 177-240.

3. European Helicobacter pylori Study Group (1997) Current European concepts in the management of Helicobacter pylori infection. The Maastricht Consens us Report. Gut 41: B-13.

4. A. esculentus L. Moench (okra): An in vitro study. Clin Microbial 2: 132. doi: 10.4172/2327-5073.1000132

5. Singh JS (2002) The Biodiversity crisis. A multifaceted review. Curr Sci 82: 638.

6. Ranabir C, Mohanty JP, Bhuyan NR, Kar PK, Nat LK (2006) Medicinal plants used against gastrointestinal tract dis orders by the healers of Sikkim Himalayas. Indian J Tradit Knowl 6: 307-311.

7. Oyelade OJ, Ade-Ohmwaje BIO, Adeomi VF (2003) Influence of variety on protein, fat contents and some physic al charasteristics of okra seeds. J Food Eng 57: 111-114.
10. Mars B (2004) Raw some: Maximizing Health, Energy, and Culinary Delight with the Raw Foods Diet, Basic Health Publications, Inc.

11. Arapitsas P (2008) Identification and quantification of polyphenolic compounds from okra seeds and skins. Food Chem 110: 1041-1045.

12. Adelakun OE, Oyelade OJ, Ade-Omowaye BI, Adeyemi IA, Van de Venter M (2009) Chemical composition and the antioxidative properties of Nigerian Okra Seed (Abelmoschus esculentus Moench) Flour. Food Chem Toxicol 47: 1123-1126.

13. Yoga C, Kumar EP, Manisha B, Hardik RM, Yamshi krishna BA (2011) An Evaluation of Antibacterial Activity of Abelmoschus esculentus on Clinically Isolated Infectious Disease Causing Bacterial Pathogen from Hospital. Int J Pharm Phytopharmacol Res 1: 107-111.

14. Carla CCCR, de Carvalho PAC, da Fonseca a MR, Xavier-Filho L (2011) Antibacterial properties of the extract of Abelmoschus esculentus. Biotechnol\ Bioprocess Eng 16: 971-977.

15. Lengsfeld C, Tiltgenmeyer F, Faller G, Hensel A (2004) Glycosylated compounds from okra inhibit adhesion of Helicobacter pylori to human gastric mucosa. J Agric Food Chem 52: 1495-1503.

16. Lengsfeld C, Faller G, Kun AH, Oyelade OJ, Ade-Omowaye BIO, et al. (2007) Okrapolyis acharides inhibit adhesion of Campylobacterjejuni to mucosa isolated from poultry in vitro but not in vivo. Anim Feed Sci Technol 135: 113-125.

17. Hunt RH, Xiao SD, Megraud F, Leon-Barua R, Bazzoli F, et al. (2011) Helicobacter pylori in developing countries. World Gastroenterology Organisation Global Guideline. J Gastrointestin Liver Dis 20: 299-304.

18. Harborne JB (1998) Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. London, Chapman and Hall.

19. Adeniyi BA, Odelola HA, Oso BA (1996) Antimicrobial potentials of Diospyros mespiliformis (Ebenaceae). Afr J Med Med Sci 25: 221-224.

20. Adeniyi BA, Onwubuc he BC, Anyiam FM, Etundayo O, Mahady GB (2009a) Anti-Helicobacter pylori activities of Eucalyptusgrandis. Effects on susceptibility, urease activity and cell surface hydrophobicity. Pharm Biol 47: 13-17.

21. Adeniyi CB, Lawal TO, Mahady GB (2009) In vitro susceptibility of Helicobacter pylori to extracts of Eucalyptus camaldulensis and Eucalyptus torelliana. Pharm Biol 47: 99-102.

22. Iwu MW, Duncan AR, Okunji CO (1999) New antimicrobials of plant origin. Perspectives on New Crops and New Uses. Alexandria, VA, ASHS Press.

23. Sofowora A (1995) Recent trends in research into African medicinal plants. J Ethnopharmacol 38: 209-214.

24. Rotimi VO, Laughon BE, Bartlett JG, Mosadomi HA (1988) Activities of Nigerian chewing stick extracts against Bacteroides gingivalis and Bacteroides melaninogenicus. Antimicrob Agents Chemother 32: 598-600.

25. Tyler VE (1999) Phytochemicals: back to the future. J Nat Prod 62: 1589-1592.

26. Mansouri S, Forouamadi A, Ghaneei T, Ahmad GN (2001) Antibacterial activity of the crude extracts and fractionated constituents of Myrtus communis. Pharma Biol 39: 399-401.

27. Ndulkwe IG, Amupitan JO, Isah Y, Adegoke KS (2007) Phytochemical and antimicrobial screening of the crude extracts from the root, stem bark and leaves of Vitellaria paradoxo. Afr J Biotech hnl 6: 1905-1909.

28. Junaid SA, Abubakar A, Ofoide AC, Olabode AO, Echeonwu GON, et al. (2008) Evaluation of Sec uridaca longipenduculat a leaf and root extracts f or antimicrobial activities. Afr J Microbiol Res 2: 322-325.

29. Chaichanawongsaroj N, Amonyorningcharoen S, Pattiyathenee P, Vlachichone R, Poovorawan Y (2012) Anti- Helicobacter pylori and anti-internalization activities of Thai folk remedies used to treat gastric ailments. J Med Plants Res 6: 1389-1393.

30. Furutogawa K, Hayashi S, Shimomura H, Yoshida T, Hatan T, et al. (2004) Antibacterial activity of hydrolyzable tannins derived from medicinal plants against Helicobacter pylori. Microbiol Immunol 48: 251-261.

31. Ferreira AC, Isomoto H, Moriyma M, Fujikoa T, Machado JC, et al. (2008) Helicobacter and gastric malignancies. Helicobacter 13: 28-34.

32. O’Connor A, Gisbert JP, McNamara D, O’Morain C (2010) Treatment of Helicobacter pylori infection 2010. Helicobacter 15: 46-52.

33. Suk KT, Baik SK, Kim HS, Park SM, Paeng KJ, et al. (2011) Antibacterial effects of the urushiol component in the sap of the lacquer tree (Rhus verniciflua Stokes) on Helicobacter pylori. Helicobacter 16: 434-443.