

Evaluation of Antimicrobial Activities of *Carica papaya*, *Azadirachta indica* and *Moringa oleifera* on *Helicobacter Pylori* Isolated from Ulcer Patients in Ondo State, Nigeria

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Abstract: Antimicrobial activities of *Carica papaya*, *Azadirachta indica* and *Moringa oleifera* on *Helicobacter pylori* isolated from ulcer patients in Ondo State, Nigeria were investigated. Hot water, cold water and two organic solvents (Pet ether and Ethanol) were used for the extraction with the highest yield being the ethanolic extracts of *Carica papaya* with 17.2%, while cold water extract of both *Carica papaya* and *Azadirachta indica* (Neem) extracts had 15.6% and 11.6% respectively. The result shows that the *Carica papaya*, *Azadirachta indica* and *Moringa oleifera* leaves extract had antimicrobial effect on the *H. pylori* isolated from ulcer patients in Ondo State, Nigeria. *Moringa oleifera* leaves showed some remarkable effect on *H. pylori* with the ethanolic extract of *Carica papaya* being the most potent. In comparison, the ethanolic extract had the highest level of antimicrobial activity than pet ether, cold and hot water extracts. The results of this study showed that leaf extracts of *C. papaya*, *A. indica* and *M. oleifera* had a very high microbial action on the isolates. The results of this study clearly suggest that leaf extracts of *C. papaya*, *A. indica* and *M. oleifera* leaves act as potent growth inhibitor of *H. pylori*. The emergence of resistance of microorganism to current first line therapy required vigorous research for substitute antimicrobial. The plants used in this study could serve as qualified plants in drug production. There is need for vigorous research to be done on the purification of these crude extracts for the development of new antibiotics to combat the infections caused by these resistant strains.

Keywords: Antimicrobial, *Carica Papaya*, *Azadirachta Indica*, *Moringa Oleifera*, *Helicobacter Pylori*, Ulcer

1. Introduction

An ulcer is basically an inflamed break in the skin or in the mucus membrane lining the gastrointestinal tract [1]. Peptic ulcer is one of the most common disorders of the gastrointestinal system which causes discomfort to the patients, disrupting their daily routine and causes mental distress [2].

H. pylori and exogenous agents such as non-steroidal anti-inflammatory drugs (NSAIDs) interact in complex ways to cause an ulcer [3]. About 70% of patients with peptic ulcer diseases are infected by bacterium *H. pylori* [4]. Up to 60% of peptic ulcers are associated with *H. pylori* infection of the stomach. Last two decades have witnessed introduction of a

number of new drugs for the treatment of peptic ulcers. None of these drugs are free from toxicities. Efforts have also been made to find suitable alternative remedies from plant and animal origins for the treatment of peptic ulcer [3].

Carica papaya (known in Ayurveda as Erand-karkati) is well known for its medicinal properties [5]. Traditionally, different parts of the papaya plant are used in the treatment of various ailments such as asthma, ulcers, eczema, diabetes, helminth infections and fever [6]. Several medicinal plants including *Carica papaya* have been reported to possess anti-ulcerogenic activity by virtue of their predominant effect on mucosal defensive factors. These plants are used to treat different gastrointestinal illnesses, including peptic ulcer disease [7].

Azadirachta indica popularly known as neem (Hindi), is a medicinal plant that grows freely all over Indian subcontinent. Neem has a role in the treatment of disorders like microbial infections, skin diseases, dental disorders, malaria, syphilis, leprosy and has antiseptic property [8]. Anti-inflammatory, immunostimulant and anti ulcerogenic actions have also been reported in the extracts of *A. indica*.

Moringa oleifera, commonly known as drumstick tree, is the most widely cultivated variety of the genus *Moringa*. It is an exceptionally nutritious vegetable tree with a variety of potential uses. Its leaves are highly nutritious and are full of medicinal properties. It finds its use as an anti-pyretic, anti-spasmodic, anti-hypertensive, anti-anemic etc and is also used for various bacterial, fungal and viral infections. In a preliminary investigation *M. oleifera* is proved to be an anti ulcerogenic [9].

Recent studies on *M. oleifera* indicate it possess antioxidant property [10]. A number of studies have implicated the role of oxidative stress in the pathophysiology of peptic ulcer [11]. Thus *M. oleifera* could be expected to play a promising role in the treatment of peptic ulcer [12].

The aim of this study was to evaluate the antimicrobial activities of *C. papaya*, *A. indica* and *M. oleifera* on *Helicobacter pylori* isolated from ulcer patients in Ondo State, Nigeria.

2. Materials and Methods

2.1. Description of Study Population

This research work was conducted in different hospitals across Ondo State, Nigeria. The eighteen (18) local government areas in Ondo State includes Akure South, Akure north, Ondo west, Ondo East, Akoko North west, Akoko North East, Akoko South East, Akoko South West, Owo, Ose, Ore, Ilaje, Ifedore, Odigbo, Okitipupa, Ikare, and Idanre. Ondo state covers an area of 15,195.2 square kilometers and lies at latitude 7° 10' North and longitude 5°05' east. A total of 355 suspected ulcer patients which include infants (1 – 9 years), 60 years and above were included in this study. Patients on ulcer medication or any other antibiotics within 7 days prior to specimen collection were excluded. Informed consent was obtained from the suspected patients and asked to fill a questionnaire provided prior to specimen collection. Ethical approval for the study was obtained from the Ethics and Research Committee of the various government hospitals across Ondo State.

2.2. Collection of Samples

The Clinical Specimens were obtained among suspected cases of ulcer patients from the selected hospitals in Ondo State, which includes; State Specialist Hospital Akure, Miteda Hospital Akure, and Ikaramu-Akoko Health Centre between June 2014 to February 2016 and were retrospectively studied. A 3g of stool samples were collected and put onto the *H. pylori* portagerm, a semisolid agar medium and transported in a cold ice packs bag at 4°C. Prior to this, the patients had been tested positive to *H.*

pylori infection using urease breath test and stool antigen test kits respectively.

2.3. Isolation of Bacteria

In isolating bacteria from the clinical specimens collected, one ml of each samples were aseptically collected and aseptically transferred into sterile McCartney bottle containing 9ml of sterile distilled water. Serial dilution were made to the fifth dilution factor and plated out on nutrients agar, Eosin methylene blue agar and Columbia blood agar plates. All the plates were incubated and observed for growth after 24hrs at 37°C. Colonies were counted and recorded as colony forming units per gram (cfu/g)

2.4. Preparation and Isolation of *H. Pylori* Inoculum

The stool samples collected were homogenized with peptone buffer solution (PBS) (250mg in 1ml PBS), then sieved the slurry with 0.25mm Millipore filter and centrifuge at 20,000xg for 30 minutes. The pellets were washed in PBS while the resulting pellets were plated out on Columbia blood agar reconstituted with Dent's medium, incubated at 37°C under microaerophilic conditions for 3-7 days.

The pure stock cultures of each isolates were scrapped from the plates and transferred onto blood agar slants and maintained at 4°C.

2.5. Standardization of Inoculum

One percent (1%) of solution of H₂SO₄ was prepared by adding 1 ml of concentrated sulphuric acid to 99 ml of distilled water, after which the solution was mixed properly. Also, 1% solution of barium chloride was prepared by dissolving 0.5g dehydrated barium chloride (BaCl₂·2H₂O) in 50 ml of distilled water. A 0.6 ml aliquot of Barium Chloride solution was added to 99.4 ml of the 1% sulphuric acid solution and it was then thoroughly mixed together. The solution was transferred in to covered tube of the same type used for both the control and the test inoculum. The solution was kept at 4°C [13].

2.6. Collection and Identification of Plant Samples

Fresh leaves of *Azadirachta indica* A. Juss (neem) were collected from the premises of Ekiti State University Teaching Hospital Ekiti State Nigeria in December 2015, *Carica papaya* Linn (Pawpaw) leaves and *Moringa oleifera* Linn were both collected at Ijapo Housing Estate Akure, Ondo State in January 2016. The leaves were authenticated at Herbarium, Forestry Research Institute of Nigeria, Ibadan in Oyo State.

2.7. Preparation of Plant Extracts

The plant extracts were prepared according to method described by Harborne [14] with slight modifications. The leaves were cleaned, cut up to small pieces and air dried for four weeks. The dried leaves were later milled using grinder and soaked in four different solvents; 80% ethanol, petroleum ether, cold water and hot water. 100g portion each of the powdered samples were soaked in one litre of each solvent, while solutions

were allowed to stand for 72hours after which they were sieved with a muslin cloth and filtered using Whatman No 1 filter paper. However, the filtrate were collected in a beaker and concentrated to dryness under reduced pressure and controlled temperature (50-55°C) to obtain solvent-free semisolid extracts. The extracts obtained were then reconstituted with 20% tween 20 (10%v/v) prior to use and sterilized with the aid of Millipore membrane filter (0.22µm). The dry weights of the extracts were recorded and measured using weighing balance and reconstituted with 20% tween 20 (10%v/v), after the reconstitution, the reconstituted extracts was used for the antimicrobial assay [15].

2.8. Antibacterial Susceptibility Testing of Plant Extracts

An agar well diffusion technique described by Ogundare and Onifade [16] was adopted to determine the *in vitro* antibacterial activity of the crude extract. A 1 ml of aliquot of 18hours broth culture that had been adjusted to the 0.5 McFarland standards were dispensed into sterile Petri dishes. Molten sterile Muller Hinton agar with dent's supplement was aseptically poured into the plates and the plates were gently rotated for organisms to homogenously distributed in the medium. The agars were allowed to solidify, after which a sterile cork borer of 6mm in diameter was used to cut uniform wells in the agar plates. The wells were then filled with 0.5ml of each extracts. In addition 20% Tween 20 (10%v/v) was used as a negative control while Chloramphenicol served as the positive control. The experiment

was conducted in triplicate and incubated at 37°C for 72hours. Clear zones around the wells were measured and recorded in millimeters.

2.9. Determination of Minimum Inhibitory Concentration (MIC) of the Plant Extracts

The extract that showed antimicrobial activity were reconstituted by diluting 0.5g of each in 10ml of 20% tween 20 (10%v/v) and then sterilized by passing through sterile Millipore membrane filter (0.45µl). However, different concentration of the extracts (50, 25, 12.5, 6.25, 3.125mg/ml) were used, while the reconstituted extracts were serially diluted in sterile broth culture and 0.1ml of the 18hours broth culture of each of the test organisms that have been adjusted to turbidity equivalent to 0.5 McFarland standard was introduced to each test tube containing the serial diluted extract and incubated for 72hours at 37°C. The tube with the least concentration of extract without growth after incubation was taken and recorded as the minimum inhibitory concentration [17].

2.10. Statistical Analysis of Data

All experiments were carried out in triplicate. Data obtained were subjected to analysis of variance and chi-square (χ^2) test using the SPSS version 21 at $P \leq 0.05$ level of significance, and treatment means were compared using Duncan's New Multiple Range Test (DNMRT).

Table 1. Percentage yield of *Carica papaya*, *Azadirachta indica* (Neem) and *Moringa oleifera* leaf Extracts.

Extracts	Initial weight of Leaves extracted (g)	Dried weight of extract recovered (g)	% recovery (g)
<i>C. Papaya</i>			
Hot water	100	11.2	11.2
Cold water	100	15.6	15.6
Pet ether	100	2.3	2.3
Ethanol	100	17.2	17.2
<i>A. indica</i>			
Hot water	100	9.7	9.7
Cold water	100	11.6	11.6
Pet ether	100	3.5	3.5
Ethanol	100	10.3	10.3
<i>M. oleifera</i>			
Hot water	100	9.1	9.1
Cold water	100	7.2	7.2
Pet ether	100	1.5	1.5
Ethanol	100	8.4	8.4

Table 2. Antibacterial effects of *Carica papaya* extracts on some isolated *H. pylori*.

Orgs	Hot water	Cold water	Pet-ether	Ethanol	Chloramphenicol
A ₁	0.00±0.00 ^a	2.33±0.33 ^b	0.00±0.00 ^a	11.00±0.33 ^c	26.67±0.33 ^d
A ₂	0.00±0.00 ^a	2.33±0.33 ^b	2.00±0.33 ^b	9.33±0.33 ^c	24.33±0.33 ^d
B ₁	3.67±0.33 ^b	5.33±0.33 ^c	0.00±0.00 ^a	11.33±0.33 ^d	24.33±0.33 ^e
B ₂	11.33±0.33 ^d	3.00±0.33 ^b	0.00±0.00 ^a	10.33±0.33 ^c	29.67±0.33 ^e
C ₁	2.67±0.33 ^b	0.00±0.00 ^a	0.00±0.00 ^a	5.33±0.33 ^c	21.00±0.33 ^d
C ₂	4.33±0.33 ^b	0.00±0.00 ^a	0.00±0.00 ^a	7.00±0.33 ^c	23.33±0.33 ^d

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter (s) along the same row are not significantly different ($P < 0.05$)

Legend; A₁ = *H. pylori* isolated from State Specialist Hospital Akure

A₂ = *H. pylori* isolated from Miteda Hospital Akure

B₁ and B₂ = *H. pylori* isolated from Ikaramu Akoko Health Centre

C₁ and C₂ = *H. pylori* isolated from State Specialist Hospital Ondo

Table 3. Antibacterial effects of *Azadirachta indica* extracts on some isolated *H. pylori*.

Org	Hot water	Cold water	Pet-ether	Ethanol	Chloramphenicol
A ₁	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	5.33±0.33 ^b	26.00±0.33 ^c
A ₂	3.33±0.33 ^b	2.67±0.33 ^b	0.00±0.00 ^a	4.33±0.33 ^c	24.33±0.33 ^d
B ₁	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	6.00±0.00 ^b	24.67±0.33 ^c
B ₂	1.67±0.33 ^b	2.00±0.33 ^c	0.00±0.00 ^a	9.33±0.33 ^d	29.33±0.33 ^e
C ₁	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	2.33±0.33 ^b	21.33±0.33 ^c
C ₂	0.00±0.00 ^a	1.33±0.33 ^b	0.00±0.00 ^a	3.33±0.33 ^c	23.33±0.33 ^d

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter (s) along the same row are not significantly different (P<0.05)

Legend; A₁ = *H. pylori* isolated from State Specialist Hospital Akure

A₂ = *H. pylori* isolated from Miteda Hospital Akure

B₁ and B₂ = *H. pylori* isolated from Ikaramu Akoko Health Centre

C₁ and C₂ = *H. pylori* isolated from State Specialist Hospital Ondo

Table 4. Antibacterial effects of *Moringa Oleifera* extracts on some isolated *H. pylori*.

Orgs	Hot water	Cold water	Pet-ether	Ethanol	Chloramphenicol
A ₁	9.33±0.33 ^d	3.67±0.33 ^b	0.00±0.00 ^a	7.33±0.33 ^c	26.33±0.33 ^e
A ₂	15.33±0.33 ^c	5.67±0.33 ^b	3.00±0.33 ^a	4.67±0.33 ^b	24.33±0.33 ^d
B ₁	13.33±0.33 ^c	2.33±0.33 ^a	2.33±0.33 ^a	6.33±0.33 ^b	24.33±0.33 ^d
B ₂	7.33±0.33 ^b	5.33±0.33 ^a	8.33±0.33 ^{bc}	9.33±0.33 ^c	29.67±0.33 ^d
C ₁	0.00±0.00 ^a	7.33±0.33 ^b	6.00±0.33 ^b	11.33±0.33 ^c	21.33±0.33 ^d
C ₂	0.00±0.00 ^a	6.33±0.33 ^b	7.33±0.33 ^c	8.33±0.33 ^d	23.33±0.33 ^e

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter (s) along the same row are not significantly different (P<0.05)

Legend; A₁ = *H. pylori* isolated from State Specialist Hospital Akure

A₂ = *H. pylori* isolated from Miteda Hospital Akure

B₁ and B₂ = *H. pylori* isolated from Ikaramu Akoko Health Centre

C₁ and C₂ = *H. pylori* isolated from State Specialist Hospital Ondo

Table 5. Minimum inhibitory concentration (mg/ml) of *Carica papaya*, *Azadirachta indica*, *Moringa oleifera*.

Orgs	Hot Water	Cold Water	Pet Ether	Ethanol	Hot Water	Cold Water	Pet Ether	Ethanol	Hot Water	Cold Water	PetEther	Ethanol
A ₁	NI	3.125	NI	12.5	NI	NI	NI	6.25	12.5	3.125	NI	12.5
A ₂	NI	3.125	6.25	3.125	3.125	6.25	NI	3.125	12.5	12.5	3.125	3.125
B ₁	3.125	3.125	NI	12.5	NI	NI	NI	3.125	12.5	3.125	3.125	12.5
B ₂	12.5	6.25	NI	12.5	3.125	3.125	NI	12.5	6.25	12.5	12.5	12.5
C ₁	3.125	NI	NI	3.125	NI	NI	NI	3.125	NI	12.5	6.25	6.25
C ₂	3.125	NI	NI	3.125	NI	3.125	NI	6.25	NI	12.5	12.5	12.5

Key; A₁ = *H. pylori* isolated from State Specialist Hospital Akure

A₂ = *H. pylori* isolated from Miteda Hospital Akure

B₁ = *H. pylori* isolated from Ikaramu Akoko Health Centre

B₂ = *H. pylori* isolated from Ikaramu Akoko Health Centre

C₁ = *H. pylori* isolated from State Specialist Hospital Ondo

C₂ = *H. pylori* isolated from State Specialist Hospital Ondo

NI= No inhibition

4. Discussion

The aim of this study was to evaluate the antimicrobial activities of *Carica papaya*, *Azadirachta indica* and *Moringa oleifera* on *Helicobacter pylori* isolated from ulcer patients in Ondo State, Nigeria. *H. Pylori* has however been presented by Smith *et al.* [18] as a major organism responsible for peptic ulcer. Transmission of the aetiological agents is mostly ascribed to fecal contamination of food and water. In developing countries a combination of untreated water, crowded conditions and poor hygiene contributes to higher occurrence of *H. pylori* infection [19].

Hot water, cold water and two organic solvents (Pet ether and Ethanol) were used for the extraction with the highest yield being the ethanolic extracts of *Carica papaya* with

17.2%, while cold water extract of both *Carica papaya* and *Azadirachta indica* (Neem) extracts had 15.6% and 11.6% respectively. In this study, various recovery percentage were observed in the study plant extracts which may have occurred due to various solvents used in the course of extraction as reported by Kordali *et al.*, [20] and Srinivasan *et al.*, [21] that individual solvents have various extraction capacities. Highest percentage recovery was observed in ethanolic extract of *Carica papaya* leaves which may be as a result of the polar bonds present in ethanol which is more active in extracting plants metabolite. This is accordance with Campos *et al.*, [22] who reported that polar solvents have been shown to be more effective in extracting organic and inorganic materials from plants. It was observed that the *Carica papaya*, *Azadirachta indica* and *Moringa oleifera* leaves

extract had antimicrobial effect on the *H. pylori* isolated from ulcer patients in Ondo State, Nigeria.

Moringa oleifera leaves showed some remarkable effect on *H. pylori* with the ethanolic extract of *Carica papaya* being the most potent. In comparison, the ethanol extract had the highest level of antimicrobial activity than pet ether, cold and hot water extracts. Das *et al.* [3] reported that the *M. oleifera* extract showed comparable antiulcer effects to the standard drug in pylorus ligation and ibuprofen induced ulcers. *Moringa oleifera* being cheap, less toxic, widely used and easily available, might play as an adjunct to the existing drugs in the pharmacotherapy of peptic ulcer. Ezikel *et al.*, [23] reported efficacy of *Carica papaya* in gastric ulcer. Kiranmai *et al.*, [24] submitted that RBM extract of *A. indica* has shown strong and promising anti-*H. pylori* activity. In actual fact, antibiotics were highly effective more than plant extracts in inhibiting test organisms due to purity level of commercial antibiotics.

5. Conclusion

The results of this study showed that leaf extracts of *C. papaya*, *A. indica* and *M. oleifera* had a very high microbial action on the isolates. The present study also looked into crude extracts that can be used in the treatment of ulcer disease while further study needs to be done on the purification of these crude extracts for the development of new antibiotics to combat the infections caused by these resistant strains.

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