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Therapeutic Potentials of Some Selected Medicinal Plant Extracts against *Helicobacter pylori* Infection: *In Vivo* Activities and Implications

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Abstract

Helicobacter pylori infection is the major cause of gastrointestinal diseases worldwide, and the high resistance of different strains against antibiotics has necessitated the search for alternative agents. Medicinal plants have shown promising results in treating various ailments, but their *in vivo* efficacy against *H. pylori* infection requires further investigation. The study focused on the in vivo activity of some selected mixtures of medicinal plants against *H. pylori* infection. A total of 186 stool and blood samples were collected and screened for HP using Columbia agar supplemented with minor nutrients. The isolates were characterized using their morphological, biochemical and molecular properties. The phytochemical constituents of *Zingiber officinale* (ZO) rhizome, *Hunteria umbellate* (HU) leaves and *Neubouda laevis* (NL) leaf extracts were determined using gravimetric and spectrophotometric methods. The therapeutic potential of the extracts was carried out using *in vivo* method. Analysis of variance (ANOVA) and student "t" test were used to analyze the data generated from the study at 95 % confidence level. *H. pylori* strain K154 (HPK154), *H. pylori* strain BS07 (HPBS07), *H. pylori* strain K93 (HPK93) and *H. pylori* strain K115 (HPK115) were mostly encountered in the study. Alkaloids, saponins, phenolics, flavonoids, tannins, and glycosides were the major phytochemicals significantly ($P<0.05$) detected in the plant extracts. The *in vivo* treatment using the extracts significantly ($P<0.05$) reduced the clinical manifestation of the pathogens, and HU+NL+ZO augmented with levofloxacin showed the highest healing activity. Therefore, the study has shown that ZO, HU and NL had significant therapeutic activities against strains of HP, and HU+NL+ZO augmented with levofloxacin was most effective.

Keywords: *Helicobacter pylori, medicinal plants, in vivo therapy, phytochemicals, antibiotic resistance*.

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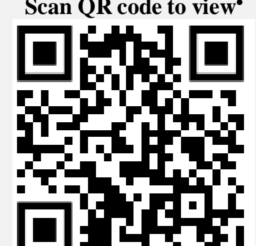

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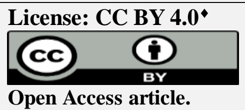

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Introduction

Helicobacter pylori is a spiral-shaped, Gram-negative, microaerophilic bacterium that colonizes the human gastric mucosa. It has been identified as the primary causative agent of chronic gastritis, duodenal and gastric ulcers, and is strongly associated with gastric cancer and mucosa-associated lymphoid tissue (MALT) lymphoma. Despite its clinical significance, the treatment of *H. pylori* infections has become increasingly complicated due to the rising global incidence of antibiotic-resistant strains.

Research has revealed that *H. pylori* is a highly pathogenic species of the genus, which infects mostly mammals especially man (Goderska *et al*., 2018). The infection that occurs due to the presence of the bacterium in the gastrointestinal tract has been recognized globally as a threat because the high level of disorderliness of the system is experienced by the infected individuals. The most debilitating aspect of the infection is that all age groups, occupations, and genders are vulnerable (Garrido-Trevino *et al*., 2022).

Several researchers have reported that the ability of the bacterium to cause severe infection with acute clinical manifestations could be attributed to the presence of virulent factors such as adhesins, which enable the organism to attach firmly to the mucosa of the stomach and urease, which enables it to breakdown urea, releasing ammonia and carbon dioxide (Avala *et al*., 2014; Azadi *et al*., 2019).

Some researchers have reported that the ability of the pathogen to produce ammonia from urea provides conducive environment for proliferation (Spinu *et al*., 2016; Mintah *et al*., 2019). The attachment of the organism in the mucosa enables it to destroy the epithelial cells in the tissue, thereby leading to bleeding in severe cases. Some of the infected patients had excreted the organism in faeces, which also provides relevant diagnostic information (Bouhenni *et al*., 2019). The wound caused by the organism is capable of depriving an infected person of several foods, especially when prepared using pepper, as it aggravates pain.

Conventional triple and quadruple therapy regimens typically include a combination of proton pump inhibitors (PPIs) with antibiotics such as clarithromycin, amoxicillin, and metronidazole. However, the declining success rate of these therapies—partly attributed to antimicrobial resistance and patient non-compliance—has prompted researchers to explore alternative therapeutic options. Natural products, plant-derived antimicrobials, and novel antibiotic formulations are gaining attention as potential candidates for combating resistant *H. pylori* strains.

Research has revealed that bioactive components of medicinal plants in Nigeria have extraordinary healing potential for all kinds of diseases, though most of them have not been optimized. The researchers actually collaborated with the local herbalists, which had been using various kinds of medicinal plants for tackling diseases. According to their findings, the medicinal plants worked beyond their imagination, but scientific knowledge was needed on dosage-related challenges, as local herbalists were unable to accurately determine safe dosages. (Elbestawy *et al*., 2023).

Some of the plants whose bioactive potentials have been widely reported are *Ocimum gratissimum, Moringa oleifera*, *Aloe barbadensis*, *Azadirachta indica, Psidium guajava*, *Zingiber officinale* etc. (Agim *et al.,* 2017; Akinsanya *et al*., 2016). There are ancestral stories which had been told and passed from one generation to another concerning the potency of the aforementioned medicinal plants, and these stories have provided additional information to researchers on the potential of each medicinal plant for further evaluation using scientific techniques. Therefore, this study was undertaken to evaluate the effect of antibacterial formation against *Helicobacter pylori* infection using *in vitro* techniques.

Materials and Methods

Sample collection: Clinical samples of blood and stool were used for the analysis. Before the collection oral consent was obtained from participants. Blood samples were collected by vein-puncture method from the anti-cubital fossa of the hand. Four milliliters (4 mL) of blood was drawn from each participant, dispensed into a non-anticoagulated container and allowed to clot. Sterile plastic stool containers without preservatives were given to each subject and they were instructed to collect stool specimens following preclusive measures as described by (Cheesbrough, 2010). The collected samples were kept inside the cooler containing an ice pack, and the samples were transported to the laboratory for immediate analysis.

Culture and Isolation of *H. pylori:* *H. pylori* bacteria were isolated from stool samples according to the method described by Umeaku *et al*. (2022) using pre-enrichment in Columbian Agar broth (Oxoid, England), with selective antibiotic (Trimethoprim, Sigma, St Louis, MO, H77883), Amphotericin B (Amresco Inc., Solar, OH, HO414), dissolved in Dimethyl-sulphoxide (DMSO) (sigma, HD5879). The stool sample was emulsified in phosphate Buffered saline and 1g of Chlorestyramine in suspension to dissolve and nullify the effect of bile in the stool as described by Ndip et al (2003). The emulsion was filtered using a sterile Muslin cloth to remove the stool debris and further filtered with a membrane filter of pore size 0.45um to retain the H. *pylori* present in the stool.

Step 1 (primary culture): as recommended by (Shahamat et al 1991);
Culture broth 1; Columbia agar-based broth (oxoid–England) was prepared according to the manufacturer's instruction, together with the following antibiotics supplements: vencomycin (10mg), Trimethoprim (4mg), Nystatin (2.5mg). 5ml aliquot was dispensed in sterile bijou bottles. The deposit on the membrane filter was cultured on the broth and incubated at the microaerophilic condition for 3-5days using an anaerobic gas park (oxoid-England) at 37˚C. This was checking immediately for the presence of visible growth (turbidity) after the first 3 days through the 12th day before discarding as no growth.

Step 2. Selective plating:
As soon as turbidity was noted, it was subcultured on *H. pylori* selective media (liophilchem, Italy) by a conventional surface-streaking technique using a sterile standard (0.02 ul) wire loop. Plates were incubated at 37˚C at microaerophilic condition for 3 to 7 days checking intermediately for growth.

Purification of the isolates: The plates that showed discrete colonies were selected and aseptically streaked each colony on sterile plates (90mm×15mm) containing nutrient agar (BIOTECH) prepared according to the manufacturer's description. The streaked plates were placed in a bacteriological incubator in inverted positions and incubated at 35 ± 2^0C for 24 h for bacteria as described in Cheesbrough (2010) and Iheukwumere *et al*. (2025a).

Characterization of the pure bacterial isolates: The pure isolates were characterized using the morphological, biochemical and molecular characteristics as described in the study published by Iheukwumere *et al.* (2018) and Iheukwumere et al. (2025b).

Preparation of plant materials: The fresh leaves of *Newbouldia laevis (Ogirisi plant*), seeds of *Hunteria Umbellata* and rhizomes of *Zingiber officinale* were collected from cultivated land at Uli in Ihiala L.G.A of Anambra State, Nigeria. The samples were appropriately authenticated and air dried under shade at room temperature for 14 days. The dried leaves were ground to powdered form using a sterile electric grinder (LXB 242/LE Max). Twenty grams of the ground samples each were macerated with distilled water and ethanol respectively for 72 h. The mixture was filtered using the Whatman No 1 filter paper. The extracts were concentrated by evaporating to dryness at room temperature in a steady air current (Iheukwumere *et al.,* 2018)

Preparation of test isolates: The test isolates were prepared using the method described by Iheukwumere *et al.* (2017). The isolates were aseptically subcultured into a broth culture and incubated at 35+ 2^0C for 24 h. The broth culture of each isolate was centrifuged using an electric centrifuge. The sediment from each culture was diluted to turbidity that matched 0.5 McFarland standard that was prepared by mixing 0.05 mL of 1.175 % $BaCl_2$ $2H_2O$ and 9.95 mL of 1 % Conc. H_2SO_4. The prepared isolates were standardized by comparing the absorbance with that of 0.5 Macfarland standards at 640 nm using a UV/visible spectrophotometer (UV1200).

Albino Wistar rats: The albino Wistar rats were purchased at the animal house, Zoology Department, University of Nigeria, Nsukka (UNN). The rats were transported to the animal house at the Department of Biochemistry, Faculty of Biosciences, Nnamdi Azikiwe University (NAU), Awka. The rats were critically examined for their weights and experimented for their suitability for the study. The rats were selected and grouped based on their weights and experiment design.

Experimented study: A total of 80 albino Wistar rats were used for this study. The rats were grouped into five groups (A, B, C, D and E). Each group contained 24 rats except group E which contained only 8 rats. The rats in groups A, B, C and D were orally administered 0.5 mL broth culture of HPK 154, HPS07, HPK 93 and HPK 115 respectively and group E was kept as a control group. The rats were allowed to feed and drink for 14 days. The infected rats (eight from each group) were then treated with Levofloxacin (1.0 mg/day) and a mixture of the extracts (HU+NL+ZO); 0.5 mL per day for 28 days. The remaining eight infected rats from groups A, B, C and D were treated with the mixture of the extracts (HU+NL+ZO) for 14 days and then treated with levofloxacin for 14 days. The rats were sacrificed after 28 days, those that had gastric ulcers were recorded, and the following analysis were carried out

Urease Test: Urease testing was performed on gastric swabs collected from the rats after scarification. The test was performed by streaking the surface of a urea agar slant tube with gastric swabs collected from rats' stomachs; place the tube in an incubator at 35°-37°C in ambient air for 48 hours to 7 days. Positive Reaction: Pink coloration in 15 minutes to 24 hours (for as long as 7 days). Negative Reaction: No change in color

Hemoglobulin investigation: Blood samples were taken from the medial canthus of the eyes after 28 days and kept in EDTA-coated tubes for haematological analysis. Hemoglobin (Hb) was determined using the Cell-Dyn 3700 Hematology Analyzer.

Statistical Analysis

The results of the data generated were expressed in percentages, tables and figures. The significance of the prevalence and susceptibility study were determined using Analysis of variance (ANOVA) at 95% confidence level. Pairwise comparison was carried out in an Excel sheet using student "t" test (Iheukwumere *et al*., 2020; Iheukwumere *et al*., 2025c).

Results

The four predominant isolates (M, N, O and P) exhibited similar cultural and morphological characteristics but differed slightly in their appearances on Columbia blood Agar and in sizes as shown in Table 1. Isolates M and P were pale grayish whereas isolates N and O were light grayish on Columbia blood Agar. The isolates were all catalase, oxidase, urease and hydrogen production positive. They fermented glucose but were negative to arabinose, lactose and maltose. They showed varied slight reactions to xylose, inositol, sorbitol and mannitol and these formed the basis of their strain variations. The sequence analysis of the bacterial isolates showed 100% identifies for all the four isolates and the identified isolates were: *Helicobacter pylori* strain K154 (HPK154, *Helicobacter pylori* strain BS07 (HPBS07) ,*Helicobacter pylori* strain K93 (HPK93) and *Helicobacter pylori* strain K115 (HPK115) as shown in Table 2

The results of the *in vivo* study are shown in Tables 3, and 4. The study revealed that urease was positive from the stomach lining of those sacrificial rats infected only with the test isolates, and later became negative after treating the infected rats with Levofloxacin (10mg/d) and HU + NL + ZO (0.5ml/d) for 21 days as shown in Table 3. The size of gastric ulcer produced among the infected rats was reduced to a low or only inflamed spot after treating the infected rats with Levofloxacin and HU + NL + ZO. It was also observed that no gastric ulcer was detected among the rats treated with HU + NL + ZO and later administered Levofloxacin as shown in Table 4. The level of hemoglobulin (Hb) was significantly ($p<0.05$) reduced among the infected rats but these were increased among the treated rats. The increase was most pronounced among the rats treated with HU + NL + ZO and later administered Levofloxacin as shown in Table 5.

Table 1: Morphological and biochemical characteristics of the isolates

Parameter	**M**	**N**	**O**	**P**
Appearance on Columbia blood agar	Pale greyish	Light greyish	Light greyish	Pale greyish
Size (mm)	1.00	0.80	0.90	1.10
Optical Nature	Translucent	Translucent	Translucent	Translucent
Edge	Smooth	Smooth	Smooth	Smooth
Surface	Smooth	Smooth	Smooth	Smooth
Gram reaction	-	-	-	-
Shape	Curved-spiral	Curved-spiral	Curved-spiral	Curved-spiral
Catalase	+	+	+	+
Oxidase	+	+	+	+
Urease	+	+	+	+
Hydrogen sulfide production	+	+	+	+
Glucose	+	+	+	+
Arabinose	-	-	+/-	-
Lactose	-	-	-	-
Maltose	-	-	-	-
Xylose	-	-	-	+/-
Inositol	+/-	-	+/-	-
Sorbitol	+/-	-	-	-
Mannitol	+/-	-	+/-	-

Table 2: Molecular identities of the bacterial isolates

Isolate	Maximum score	Total score	Query Cover	E-value	Identity (%)	Accession Number	Description
M	23555	23555	100	0.0	100.00	CP091771.1	*Helicobacter pylori* strain K154 (HPK154) complete genome
N	12770	12770	100	0.0	100.00	CP122947.1	*Helicobacter pylori* strain BS07 (HPBS07) complete genome
O	47493	47493	100	0.0	100.00	CP091769.1	*Helicobacter pylori* strain K93 (HPK93) complete genome
P	29676	29676	100	0.0	100.00	CP091770.1	*Helicobacter pylori* strain K115 (HPK115) complete genome

Table 3: *In vivo* study based on urease production

Experimented set-up	HPK154	HPBS07	HPK93	HPK115
Infected group	+	+	+	+
Levofloxacin (Lev/10mg/d)	-	-	-	-
HU + NL + ZO (0.5ml/d)	-	-	-	-
HU + NL + ZO * Lev	-	-	-	-

Mg/d – Milligram per day
Lev / HU + NL + ZO – Administered for 28 days after infection
HU + NL + ZO * Lev – Administered Extract for 14 days after infection, and later administered Lev for 14 days.

Table 4: *In vivo* study based on gastric ulcer formation

Experiment set-up	HPK154	HPBS07	HPK93	HPK115
Infected Group	++	+++	+++	+++
Levofloxacin (Lev/10mg/d)	-	+/-	+	+
HU + NL + ZO (0.5ml/d)	-	+/-	+/-	+/-
HU + NL + ZO * Lev	-	-	-	-

Mg/d – Milligram per day
Lev / HU + NL + ZO – Administered for 28 days after infection
HU + NL + ZO * Lev – Administered Extract for 14 days after infection, and later administered Lev for 14 days.
+++ = high; ++ = moderate
+ = low; +/- = inflammation

Table 5: *In vivo* study based on hemoglobulin (Hb) level

Experimented set-up	Hb (g/dl)			
	HPK154	HPBS07	HPK93	HPK115
Infected group	9.26 ± 0.10	8.84 ± 0.11	9.47 ± 0.10	9.14 ± 0.13
Lev (10mg/d)	11.26 ± 0.17	10.98 ± 0.14	11.34 ± 0.10	11.16 ± 0.17
HU + NL + ZO (0.5ml/d)	12.64 ± 0.14	11.07 ± 0.10	12.86 ± 0.10	12.42 ± 0.13
HU + NL + ZO * Lev	12.96 ± 0.17	13.24 ± 0.11	13.07 ± 0.21	13.11 ± 0.11

Mg/d – Milligram per day
Lev / HU + NL + ZO – Administered for 28 days after infection
HU + NL + ZO * Lev – Administered Extract for 14 days after infection, and later administered Lev for 14 days.

Discussion

The characteristics and identities of different strains of *H. pylori* encountered in both stool and blood samples are in line with the reports of many researchers (Egwu and Chukwubike, 2014; Lopes *et al*., 2014; El-Shabrawi *et al*., 2018; Nwachukwu *et al*., 2020; Hussein *et al*., 2021). *H. pylori* strain K154 (HPK154), *H. pylori* strain BS07 (HPBS07), *H. pylori* strain K93 (HPK93) and *H. pylori* strain K115 (HPK115) were encountered in the studied samples. Man researchers (El-Shabrawi *et al*., 2018; Nwachukwu *et al*., 2020; Hussein *et al*., 2021) encountered *H. pylori* from their studied samples but with varied strains.

The pronounced activity of those infected rats initially treated with HU + NL + ZO and later treated with Levofloxacin supported the plasmid eviction potentials of the extracts mixture and the therapeutic of the antibiotics as reported.
Several researchers (Krzyzek *et al*., 2021; EL-Sherbinv *et al*., 2022; Elbestawy *et al*., 2023) reported the in vivo activities of different medicinal plants against *H. pylori*. Bi *et al*. (2014) reported that the bactericidal activity of plant preparations against *H. pylori* significantly depends on the type of the extract and its components, concentration and exposure time, and the density of the tested bacterial strains. The tendency to monitor the amount of urease and other virulence factors for evaluation of in vivo activities of some medicinal plants against *H. pylori* corroborated with the findings of Krzyzek *et al*. (2021), who investigated the effect of various compounds on bacterial virulence factors such as urease, adhesion, vacuolization, motility or on specific metabolic enzymes. Ayala *et al*. (2014) reported that phenols are the largest group of metabolites in the studied plants and many compounds are believed to have remarkable anti-*H. pylori effect in vitro* and, to some extent, *in vivo*.

Conclusion

The study revealed that *Helicobacter pylori* strain K154 (HPK154), *H. pylori* strain BS07 (HPBS07), *H. pylori* strain K93 (HPK93) and *H. pylori* strain k115 (HPK115) were encountered in the studied stool and blood samples. The study further revealed that the mixture of the rhizomes of *Zingiber officinale* (ZO), leaves of *Hunteria umbellata* (HU) and *Neuboudia laevis* extracts exhibited significant therapeutic activity against H. pylori, and the mixture augument with Levofloxacin showed the best *in vivo* activity in the present study.

Conflict of interests: The authors declare that they have no conflict of interests.

Authors Contributions: All authors contributed towards the study design, experiment execution, data analysis, and manuscript drafting.

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