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Medicinal Plant Extracts Enhance Conventional Antibiotics Activity against *Helicobacter pylori*: An *In Vitro* Assessment

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Abstract

In Nigeria, the proliferation of resistance strains associated with *H. pylori* has complicated treatment regimens, combination therapies, including natural products, may offer a promising approach to enhance treatment efficacy. This study x-rayed the effect of conventional antibiotics on H. pylori which was exposed to some selected medicinal extracts. 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 *in vitro* activities of medicinal plants against the isolates were carried out using the Agar-welled diffusion method. Exposure of the resistance strains to different concentrations of the best mixture of extracts (HU+NL+ZO) was carried out using the tube dilution technique. The disk technique was used to examine the susceptibility of the exposed strains to conventional antibiotics. Analysis of variance (ANOVA) and student "t" test were used to analyze the data generated from the study at a 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. ZO, HU and NL extracts significantly ($P<0.05$) reduced the number of resistance strains from 36.82 % to 0.14 %, and NL+HU+ZO recorded the highest activity. Therefore, exposing HPK154, HPBS07, HPK93 and HPK115 to the studied medicinal plant extracts at their optimal level, reduced the level of resistance associated with the organism, and NL+HU+ZO was proven to be the greatest enhancer.

Keywords: *Helicobacter pylori resistance, Medicinal plant extracts, Phytochemical analysis, Antibiotic susceptibility*

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1. Introduction

The era where traditional medicine practitioners are disdained has gone because everyone has discovered that there are bioactive components in medicinal plants, which are yet to be tapped (Mintah *et al*., 2019). Therefore, relying on one source of antimicrobial agent, which had not yielded the desired goal,

would put the health of mankind in jeopardy. As a result of this, researchers in biomedical sciences have intensified efforts to isolate and study pathogenic bacteria that pose a serious threat to mankind such as *Helicobacter pylori* (Bi *et al*., 2014). *Helicobacter pylori* is a Gram-negative, micro-aerophilic and spiral-shaped bacterium that belongs to the family *Helicobacteraceae*. The genus has several species, of which some are found in the upper gastrointestinal tract and liver of some mammals and birds. The species of the genus are motile due to the presence of flagellum (Savoldi *et al*., 2018).

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.

It is appalling that for decades, most of the drugs and antibacterial agents had not yielded the desired goal. To an extent, it was speculated that stomach ulcer caused by *H. pylori* has no cure but can only be checked. As a result of this, it has been difficult to have access to a drug that can totally cure ulcer infection, especially in developing countries, where the prevalence rate remains almost constant (Alibi *et al*., 2020).

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, who had been using various kinds of medicinal plants to tackle 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. Some religious beliefs regard herbal medicine as diabolic, which has generated a lot of controversies on its acceptance in such locality, but one of the problems that biomedical researchers tend to solve is to convince people that medicinal plants are naturally endowed with bioactive agents, and it is not associated to evil forces (Akinyemi *et al*., 2017).

2. 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 C.N *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 microaerophilic conditions 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^{0}C for 24 h for bacteria as described in Cheesbrough (2010).

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).

Determination of the Susceptibility of the Test Isolates Exposed to Various Concentrations of the Extracts against Conventional Antibiotics
Preparation of broth culture: The 24 h broth culture was prepared by subculturing the test isolates in a nutrient broth prepared following the manufacturer's direction. This was incubated at 37˚C for 24 h.

Synergistic activities: This was carried out following the modified method described by Iheukwumere *et al*. (2020). A 24 h broth culture of the test isolates was prepared in test tubes (Pyrex). Then the test isolates were exposed to different concentrations (1%, 5%, 10%, 15% 20%, 25%, 30%, 35%, 40%, 45%, and 50%) of the best-formulated mixture of the plant extracts, and incubated for 24 h at 37±2^{o}C to determine the sub-lethal concentrations of the agents. After 24 h of incubation, 1-millimeter aliquot from each test tube was inoculated into a nutrient agar plate and incubated, after which colonies were selected and counted. Then the susceptibility of the cured isolates was carried out using the disk diffusion technique. Then the selected plates that had reasonable growth were used for this study. These isolates were inoculated into freshly prepared Muller Hinton agar plate. Then, antibiotic discs of prior resistance were aseptically introduced into the plate, ensuring that the discs made appropriate contact with the surface of the agar. These were incubated for 24 h at 37±2^{o}C after which plates were examined.

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).

3. 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 study revealed that after treating HPK154, HPBS07, HPK93 and HPK115 with the mixture of NL + HU + ZO, combined using the optimum concentration of their activities, the level of their resistance reduced from 33.33%, 37.50%, 40.00% and 50.00% to 0.00%, 12.50%, 0.00% and 0.00% respectively against Levofloxacin. There was significant ($p<0.05$) reduction in the level of resistance as shown in Table 3. Similarly, the mixture of the extracts reduced the level of resistance of the isolates from 77.78%, 81.25%, 75.00% and 100.00% to 22.22%, 25.00%, 25.00% and 0.00% respectively against Clarithromycin and the reduction was statistically significant ($p<0.05$) as shown in Table 4. The extract mixture reduced the resistance isolates from 100.00%, 87.50%, 100.00% and 100.00% to 11.11%, 18.75%, 40.00% and 0.00% respectively against grazone as shown in Table 5.

Table 6 and 7 revealed the reduction of the level of resistance from 100.00% in all isolates to 0.00%, 12.50%, 25.00%, and 000% respectively against amoxicillin, and 44.44%, 31.25%, 25.00% and 0.00% respectively against metronidazole.

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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) compl 550bp
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

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Table 3: Susceptibility of the treated isolates against Levofloxacin

Isolates	N	Before Treatment		After Treatment	
		S(%)	R(%)	S(%)	R(%)
HPK	9	6(66.67)	3(33.33)	9(100.00)	0(0.00)
HPBS07	16	10(62.50)	6(37.50)	14(87.50)	2(12.50)
HPK93	5	3(60.00)	2(40.00)	5(100.00)	0(0.00)
HPK115	2	1(50.00)	1(50.00)	2(100.00)	0(0.00)
Total	32	20(62.50)	12(37.50)	30(93.75)	2(6.25)

Table 4: Susceptibility of the treated isolates against Clarithromycin

Isolate	N	Before Treatment		After Treatment	
		S(%)	R(%)	S(%)	R(%)
HPK154	9	2(22.22)	7(77.78)	7(77.78)	2(22.22)
HPBS07	16	3(18.75)	13(18.25)	12(75.00)	4(25.00)
HPK93	5	1(25.00)	4(75.00)	4(75.00)	1(25.00)
HPK115	2	0(0.00)	2(100.00)	2(100.00)	0(0.00)
Total	32	6(18.75)	26(81.25)	25(78.13)	7(21.87)

Table 5: Susceptibility of the treated isolates against Grazone

Isolate	N	Before Treatment		After Treatment	
		S(%)	R(%)	S(%)	R(%)
HPK154	9	0(0.00)	9(100.00)	8(88.89)	1(11.11)
HPBS07	16	2(12.50)	14(87.50)	13(81.25)	3(18.75)
HPK93	5	0(0.00)	5(100.00)	3(60.00)	2(40.00)
HPK115	2	0(0.00)	2(100.00)	2(100.00)	0(0.00)
Total	32	3(6.25)	30(83.75)	26(81.25)	6(18.75)

Table 6: Susceptibility of the treated isolates against Amoxicillin

Isolate	N	Before Treatment		After Treatment	
		S(%)	R(%)	S(%)	R(%)
HPK154	9	0(0.00)	9(100.00)	9(100.00)	0(0.00)
HPBS07	16	0(0.00)	16(100.00)	14(87.50)	2(12.50)
HPK93	5	0(0.00)	5(100.00)	4(75.00)	1(25.00)
HPK115	2	0(0.00)	2(100.00)	2(100.00)	0(0.00)
Total	32	0(0.00)	32(100.00)	29(90.63)	3(9.37)

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Table 7: Susceptibility of the treated isolates against metronidazole

Isolate	N	Before Treatment		After Treatment	
		S(%)	R(%)	S(%)	R(%)
HPK154	9	0(0.00)	9(100.00)	5(55.56)	4(44.44)
HPBS07	16	0(0.00)	16(100.00)	11(68.75)	5(31.25)
HPK93	5	0(0.00)	5(100.00)	4(75.00)	1(25.00)
HPK115	2	0(0.00)	2(100.00)	2(100.00)	0(0.00)
Total	32	0(0.00)	32(100.00)	22(68.75)	10(31.23)

4. 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 activities of Levofloxacin against those *H. pylori* isolates exposed to various concentrations of HU + NL + ZO could be attributed to the ability of the plant extracts mixture to evict the resistance plasmid from the resistance from the resistant strains. Khare *et al*. (2021) reported that phytochemicals such as alkaloids and flavonoids have multidrug resistance reversing ability, and these phytochemicals can reverse multidrug-resistant strains by affecting the expression or activity of the ABC transports and inhibiting the plasmid replication. The bioactive molecules can intercalate plasmid DNA or chromosomal DNA and distort their replication. They also reported that phytochemicals can form synergistic interaction with antibiotics which can address the problems of other molecular targets.

5. 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 highly reduced the level of resistance associated with the isolates when exposed to conventional antibiotics.

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Availability of Data and Materials: All datasets analyzed and described during the present study are available from the corresponding author upon reasonable request.

References

Adeniyi, B. A., Otegbayo, J. A., Lawal, T. O., Oluwasola, A. O., Odaibo, G. N. & Okolo C. (2012). Prevalence of *Helicobacter pylori* infection among dyspepsia patients in Ibadan, South West Nigeria. *African Journal of Microbiology Research,* **6**(14), 3399-3402.

Agim, C. S., Nosiri, C. I., Anyanwu, C. and Atasie, O. (2017). Evaluation of the Phytochemical and GC–MS Analysis of the Aqueous Seed Extract of *Aframonum melegueta*. *Journal of Pharmacognosy and Phytochemistry* **6** (2): 101 – 104.

Akinsanya, B., Utoh, O. U. and Ukwa, U. D. (2016). Toxicological, Phytochemical and Anthelminthic Properties of Rich Plant Extracts on *Clarias gariepinus. Journal of Basic and Applied Zoology* **74**: 75-86.

Alibi, S., Crespo, D., Navas, J. (2021) Plant-Derivatives Small Molecules with Antibacterial Activity. *Antibiotics,* **10**: 231.

Ayala, G., Escobedo-Hinojosa, W.I., De La Cruz-Herrera, C.F., Romero, I. (2014). Exploring Alternative Treatments for *Helicobacter Pylori* Infection. *World Journal of Gastroenterology,* **20:** 1450.

Ayala, G., Escobedo-Hinojosa, W.I., De La Cruz-Herrera, C.F., Romero, I. (2014). Exploring Alternative Treatments for *Helicobacter Pylori* Infection. *World Journal of Gastroenterology* **20:** 1450.

Azadi, M., Ebrahimi, A., Khaledi, A., Esmaeili, D. (2019). Study of Inhibitory Effects of the Mixture of Cinnamon and Ginger Extracts on Caga Gene Expression of *Helicobacter Pylori* by Real-Time RT-PCR Technique. *Gene Reports,* **17:** 100493

Bi, W.-P., Man, H.-B., Man, M.-Q. (2014). Efficacy and Safety of Herbal Medicines in Treating Gastric Ulcer: A Review. *World Journal of Gastroenterology,* **20:** 17020.

Bouhenni, H., Doukani, K., Şekeroğlu, N., Gezici, S., Tabak, S. (2019). Comparative Study on Chemical Composition and Antibacterial Activity of Fenugreek (*Trigonella Foenum Graecum* L.) and Cumin (*Cuminum Cyminum* L.) Seeds. *Ukrainian Food Journal,* **8:** 755–767

Cheesbrough, M. (2010). District Laboratory Practice in Tropical Countries, Part 1, Second Edition. Cambridge University Press, Cambridge, pp. 333−361.

Elbestawy, M.K.M., El-Sherbiny, G.M., Moghannem, S.A. (2023). Antibacterial, Antibiofilm and Anti-Inflammatory Activities of Eugenol Clove Essential Oil Against Resistant *Helicobacter Pylori*. *Molecules,* **28:** 2448.

El-Shabrawi, M., Abd El-Aziz, N., El-Adly, T. Z., Hassanin, F., Eskander, A., Abou-Zekri, M., Mansour, H. & Meshaal, S. (2018). Stool Antigen Detection Versus 13 C-Urea Breath Test for Non-Invasive Diagnosis of Pediatric Infection in A Limited Resource Setting. *Archives of Medical Science,* **14**(1), 69 – 73.

Ezugwu, R. I. & Chukwubike, C. (2014). Epidemiology of *Helicobacter pylori* infection among dyspepsia patients in South-East, Nigeria. *IOSR Journal of Pharmacy and Biological Sciences*, **9**(6), 53-56.

Garrido-Treviño, L., López-Martínez, M., Flores-Hinojosa, J., Tijerina-Rodríguez, L., Bosques-Padilla, F. (2022). Empiric Treatment Vs Susceptibility-Guided Treatment for Eradicating *H. Pylori*: Is it Possible to Change that Paradigm Using Modern Molecular Methods? *Review of Gastroenterology*, **87:** 330–341.

Goderska, K., Agudo Pena, S., Alarcon, T. (2018). *Helicobacter Pylori* Treatment: Antibiotics or Probiotics. *Applied Microbiology and Biotechnology*, **102:** 1–7.

Hussein, R. A., Al-Ouqaili, M. T. S. & Majeed, Y. H. (2021). Detection of *Helicobacter Pylori* Infection by Invasive and Non-Invasive Techniques in Patients with Gastrointestinal Diseases from Iraq: A Validation Study. *Plos One*, **16**(8), 256 – 393

Iheukwumere, I.H, Dimejesi, S.A, Iheukwumere C.M, Chude C.O, Egbe P.A, Nwaolisa C.N, Amutaigwe E.U, Nwakoby N.E, Egbuna C, Olisah M.C, And Ifejeme J.C. (2020). Plasmid Curing Potentials of Some Medicinal Plants Against Citrate Negative Motile *Salmonella* Species. *European Journal of Biomedical and Pharmaceutical Sciences.* **7**(5), 40-47.

Iheukwumere, I.H**.** Uneze, B.C. and Ejike, C.E. (2017). Efficacy of some selected antimicrobial substances in prevention of enteric bacteria in broiler chicks. *Journal of Biology, Agriculture and Health Care* **7** (4) :58–67.

Iheukwumere, I.H., Chude, C. and Unaeze, B.C. (2018). Molecular characterization andenterotoxigenicity profiles of enteric bacteria isolated from chicken feeds. *Journal of Natural Sciences Research* **8**: 51–64

Khare, T., Anand, U., Dey, A., Assaraf, Y. G., Chen, Z. S., Liu, Z., & Kumar, V. (2021). Exploring Phytochemicals for Combating Antibiotic Resistance in Microbial Pathogens. *Frontiers in pharmacology*, *12*, 720726.

Lopes, A. I., Vale, F. F. & Oleastro, M. (2014). *Helicobacter Pylori* Infection Recent Developments in Diagnosis. *World Journal Gastroenterology*, **20**, 9299 – 9313.

Mintah, S.O., Asafo-Agyei, T., Archer, M.-A., Junior, P.A.-A., Boamah, D., Kumadoh, D., Appiah, A., Ocloo, A., Boakye, Y.D., Agyare, C. (2019). Medicinal Plants for Treatment of Prevalent Diseases. *Pharmacogenesis of Medicinal Plants*, 1–19.

Ndip R. N., MacKay W. G., Farthing M.J. G., Weaver L. T. Culturing of Helicobacter pylori from clinical specimens. Journal of Pediatric Gastroenterology and Nutrition. 2003; Volume 36 - Issue 5 - pp 616 – 622.

Nwachukwu, E. P., Onwurah, O. W., Amilo, G. I., Onwuasoanya, U. F. & Ezeugwunne, I. P. (2020). Prevalence Of *Helicobacter Pylori* Among Patients with Gastritis Attending Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria. Ann *Current Gastroenterology Report*, **1**(1), 1003 – 1010.

Savoldi, A., Carrara, E., Graham, D.Y., Conti, M., Tacconelli, E. (2018). Prevalence of Antibiotic Resistance in *Helicobacter pylori*: A Systematic Review and Meta-Analysis in World Health Organization Regions. *Gastroenterology*, **155**: 1372–1382.

Shahamat, M., Paszko-Kolva, C., Keiser, J., & Colwell, R. R. (1991). The sequential culturing method improves recovery of Legionella spp. from contaminated environmental samples. *Zentralblatt für Bakteriologie*, *275*(3), 312-319.

Spînu, M., Niculae, M., Paştiu, A.I., Şandru, C.D., Pall, E., Vasiu, A. (2016). Vegetal Extracts Influence In Vitro On the Cell-Mediated Immunity In Carnivores Depending On Health Status, Target Species And Plant Taxonomy. *Ind. Crops Prod. 88*, 44–47.

Umeaku, C. N., Ezendianefo, J. N., Chris-umeaku, C. I., and Dimejesi, S. A. (2022). Occurrence of antibiotic-resistant bacteria and antibiotic-resistant genes in rivers found in Anambra state, Nigeria. *GSC Biological and Pharmaceutical Sciences*, 304-316.

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