

THE EFFECT OF SOME NIGERIAN LOCAL HERBS ON HELICOBACTER PYLORI

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Four Nigerian medicinal plants commonly used in the treatment of bacterial infections were tested for antimicrobial activity against twenty local strains of *Helicobacter pylori* recovered from patients with gastro-duodenal ulcers and gastritis. In vitro agar diffusion assay revealed anti-*Helicobacter pylori* activity of ethanolic extracts of *C. papaya* and *M. lucida* to 80% (16/20) of the isolates tested. While the ethanolic extracts of *O. gratissimum* and *P. amarus* inhibited the colonial growth of 35% (7/20) of these strains. The zones of inhibition ranged from 5 – 20 mm in diameter. Contrastingly, the aqueous extracts of these plants appeared to lack anti-*Helicobacter pylori* activity except in *M. lucida* and *O. gratissimum* where inhibition of a total of three isolates was observed. The present results suggest the presence of anti-*Helicobacter pylori* principles in ethanolic extracts of *C. papaya* and *M. lucida* and support their future use in the treatment of ulcers and gastritis in Nigeria.

INTRODUCTION

It is over a decade that *Helicobacter pylori* infections were known as a major cause of gastro-duodenal ulcers, gastritis and stomach cancer with greater burden of cases documented in developing countries (1, 2). Most effective therapies employ a synergic action between a gastric acid release inhibitor and one or more antibiotics to eradicate *Helicobacter pylori*, its urease and associated diseases (3, 4). However, the implementation of these therapies in communities where the poor bears the greater brunt of the disease is cost ineffective as concerned drugs are poorly patronized (5).

In Nigeria, *Helicobacter pylori* is fastly replacing non steroidal anti-inflammatory drugs (NSAIDs) as causal agent of gastro-duodenal ulcers and duodenal perforation cases are also of significant increase (6). More worrisome is the increased resistance trend of *Helicobacter pylori* isolates to metronidazole, amoxicillin and tetracycline *in vitro* (7) in a manner that discourage their future clinical use against *Helicobacter pylori* infection in the country. In Nigeria, a triple therapy involving omeperazole, metronidazole and amoxicillin are widely used in severe cases and treatment failures with this combination have been reported (8). Alternative triple

therapies that could be used are expensive, have undesirable side effects (9) and a long list of contraindications (10). The discoveries that *Allium* vegetables harbour anti-*Helicobacter pylori* substances (11,12) have further heightened the global search for similar compounds in other medicinal plants and this to some extent has yielded encouraging results that have the therapeutic applications in areas of discovery as a barrier (13). *Morinda lucida* (Rubiaceae), *Ocimum gratissimum* (Lamiaceae), *Carica papaya* (Caricaceae) and *Phyllanthus amarus* (Euphorbiaceae) extracts are among the folkloric remedies that have been confirmed scientifically to possess clinical values against protozoal and bacterial infections in Nigeria (14, 15, 16, 17). These plants grow abundantly in Nigerian soils and are not ethnically or age group biased in use. This further explains why their potentials in the future treatment of gastritis and ulcers in Nigeria are investigated.

MATERIALS AND METHOD

Plant materials

The plants, were collected from various local markets in Lagos, identified and confirmed by Mrs. B. Opere of the Department of Botany, Lagos State University. Voucher samples of these plants were subsequently deposited in

the Department. The plants used were listed in Table 1.

Extraction

A simple extraction procedure of Olukoya *et al* (18) was adopted to prepare aqueous and organic extracts of the plants tested. To prepare aqueous extracts, 1.1 g of plants' leaves (previously dried at 50°C and ground into fine powders) were steeped in 10 ml of sterile-distilled water at 30 – 32°C for five days. The organic extracts were prepared by steeping 1.2 g of plant materials in 5 ml of 40% ethanol. Extracts were then passed through Hemmings filters (BTI UK) and the resulting sterile filtrates were aseptically transferred to sterile bottles and labeled as crude extracts of individual plants. The organic extracts were subsequently reconstituted with phosphate buffered saline solution (pH 7.2) to nullify the effect of ethanol on the tested organisms. A mixture of 0.1 ml of sterile water and 5 ml of 40% ethanol was prepared as a control.

Microbial cultures

Twenty strains of *Helicobacter pylori* recovered from the biopsy samples of patients with gastritis and gastro-duodenal ulcers from Western Nigeria were used as test organisms. *Helicobacter pylori* ATCC 49503 was used as control. All organisms were cultured on Columbia agar base (Oxoid, CM331) containing 7% sheep blood.

Sensitivity Testing

Antimicrobial susceptibility testing was carried out using the agar diffusion technique. In brief, Isosensitest agar (Oxoid, UK) plates were holed (6 mm in diameter) with the aid of a sterile cork-borer and seeded with 10 µL of *H. pylori* suspension (McFarland 3). The plates were dried in the air and 100 µL of plant extract was introduced into the wells. The plates were incubated microaerophilically (5% O₂, 10% CO₂) at 37°C for 4 days. Holes containing bacterial suspension (10 µL of 9 x 10⁸ CFU/ml) and sterile water or ethanol (100 µL) were used as controls. Diameters of zones of inhibition of both the tested organisms and standard strain (*H. pylori* ATCC 45903) were measured in millimeters (mm) and recorded.

RESULTS.

Table 2 gave the summary of the antimicrobial activity of aqueous and ethanolic extracts of the plants against the twenty *H. pylori* isolates and the standard strain. The ethanolic extracts of *C. papaya*, *M. lucida*, *O. gratissimum* and *P. amarus* provided evidence of anti-*Helicobacter pylori* activity in 80% (16/20) and 30% (7/20) of the isolates tested. *Helicobacter pylori* ATCC 49503 was observed to be susceptible to the organic extracts of *C. papaya* and *M. lucida* only. The zones of growth inhibition were 5 – 20 mm in diameter. Apart from the water extracts of *M. lucida* and *O. gratissimum*, which inhibited the growth of 10% (2/20, 5 – 15 mm) and 5% (1/20, 10 – 15 mm) of the isolates tested, aqueous extracts of other plants were found susceptible to these isolates. The standard strain was resistant to all aqueous preparations. Unlike sterile water wells, no colonial growth was found in 40% ethanol control wells.

Table 1: The local herbs selected for testing

Botanical name	Local name*	Plant part tested
<i>Carica papaya</i>	Ibepe	Leaf
<i>Morinda lucida</i>	Ejirin	Leaf
<i>Octimum gratissimum</i>	Efirin	Leaf
<i>Phyllanthus amarus</i>	Ehinolubinsowo	Leaf

* Nigerian (Yoruba) names.

Table 2: Antimicrobial activity of ethanol and water extracts of the four local herbs

Strain code no	<i>C. papaya</i>		<i>M. lucida</i>		<i>O. gratissimum</i>		<i>P. amarus</i>		Sterile water	40% Ethanol
	E	W	E	W	E	W	E	W		
Hp 1	0	0	2+	0	0	0	0	0	0	2+
Hp 2	2+	0	2+	0	1+	0	0	0	0	3+
Hp 3	1+	0	2+	0	0	0	0	0	0	1+
Hp 4	2+	0	2+	2+	0	0	0	0	0	2+
Hp 5	1+	0	3+	0	2+	0	2+	0	0	3+
Hp 6	3+	0	1+	0	0	0	0	0	0	1+
Hp 7	2+	0	2+	0	2+	0	0	0	0	2+
Hp 8	0	0	0	0	0	0	0	0	0	1+
Hp 9	2+	0	0	0	0	0	0	0	0	2+
Hp 10	2+	0	2+	0	0	0	1+	0	0	2+
Hp 11	2+	0	2+	0	2+	0	2+	0	0	3+
Hp 12	1+	0	3+	0	2+	0	2+	0	0	2+
Hp 13	2+	0	1+	1+	0	0	0	0	0	3+
Hp 14	0	0	0	0	0	0	0	0	0	2+
Hp 15	2+	0	2+	0	0	2+	1+	0	0	3+
Hp 16	1+	0	2+	0	1+	0	1+	0	0	2+
Hp 17	2+	0	3+	0	0	0	0	0	0	2+
Hp 18	0	0	0	0	0	0	0	0	0	2+
Hp 19	2+	0	2+	0	1+	0	1+	0	0	3+
Hp 20	3+	0	3+	0	0	0	0	0	0	2+
Hp 49305	2+	0	2+	0	0	0	0	0	0	3+

Keys: W = water extract; E = Ethanolic extract; 0 = No inhibition; 1+ = 5 - 9 mm diameter zone of inhibition; 2+ = 10 - 15mm; 3+ = 16 - 20mm; Hp = *Helicobacter pylori* strains.

DISCUSSION

In this study, ethanolic extracts of *C. papaya* and *M. lucida* were observed to prevent the growth of 80% of *Helicobacter pylori* strains tested in vitro. Similar extracts of *O. gratissimum* and *P. amarus* also demonstrated anti-*Helicobacter pylori* activity but in only 35% (7/20) of the isolates. The lack of inhibition observed in water wells confirmed the viability of all the isolates tested. While the inhibitory effect of 40% ethanol attested to the appropriateness of the reconstitution procedure, the

poor antibacterial activity of the water extracts of these plants implies that water has inadequate power to extract anti-*Helicobacter pylori* principles from these plants. However, With respect to organic extraction, this finding has provided scientific evidence for antibacterial activity of *Morinda lucida* in vitro as most scientific findings described the plant as an anti-malaria herb (14). The study of Agomo *et al* (19), which demonstrated a complex array of cellular responses to *M. lucida*

administered to mice infected with *Plasmodium yoeli nigeriensis* seemed to provide an indication that numerous biological properties are inherent in this plant. *Ocimum gratissimum* has been extensively demonstrated to inhibit aetiologic agents of diarrhoeal *in vitro* and *in vivo* (15, 20). However, the result obtained from this study should be interpreted with caution as strains of *O. gratissimum* are characterized by varying chemical composition (21). The present study has also extended the biological functions of *Carica papaya* whose seeds have demonstrable evidence of having antifertility effects in rats (22). Although, antimalaria activity of *P. amarus* in mice and rats has been observed in the laboratory (*Personnal communication*), this is first time anti-*Helicobacter pylori* activity will be ascribed to this plant in Nigeria. Based on our findings, there is no doubt that these plants hold tremendous clinical promise especially in rural communities, which provide the greater number of patients and severe cases of gastro-duodenal ulcer. The present study is still in its infancy and therefore invite more research studies to elucidate the active anti-*Helicobacter pylori* substances in these plants, investigate synergy associated with extract combination leading

to ultimate suggestion of whether these plants can be combined with orthodox drugs to met the criteria of gastric acid suppression, *H. pylori* eradication and stomach protection in the treatment of ulcers and gastritis. In conclusion, the present study has revealed, the tremendous potentials inherent in ethanolic preparations of *C. papaya* and *M. lucida* if adopted for future treatment of ulcers and gastritis in Nigeria.

REFERENCES

1. Dixon MF. *Helicobacter pylori* and gastritis. In: Rathbone BJ, Heatley RV (eds). *Helicobacter pylori* and gastro-duodenal disease. Blackwell Scientific Publications. Oxford, UK. 1992 : 124 – 129.
2. Feldman RA, Eccersley AJ, Hardie JM. Epidemiology of *Helicobacter pylori*, acquisition, population prevalence and disease – to – infection ratio. *Br. Med. Bull.* 1998 ; **54** : 39 – 53.
3. Mansour-Ghanael F, Fallah M, Shafaghi A. Eradication of *Helicobacter pylori* in duodenal ulcer disease. Tetracycline & Furozolidone vs. Metronidazole & Amoxicillin in

- omeperazole based therapy. *Med. Sci. Monit.* 2000 ; **8** : 127 – 130.
4. Jonkers D, Broek DE, Dooren IV, et al. Antibacterial effect of garlic and omeperazole on *Helicobacter pylori*. *J. Antimicrob. Chemother.* 1999 ; **43** : 837 – 839.
 5. Drouin E. *Helicobacter pylori*: novel therapies. *Can. J. Gastroenterol.* 1999 ; **13** : 581 – 583.
 6. Smith SI, Oyedeji KS, Arigbabu O, et al. The prevalence of *Helicobacter pylori* from gastritis and ulcer patients in Western Nigeria. *Biomedical Letters.* 1999 ; **60** : 115 – 120.
 7. Smith SI, Oyedeji KS; Arigbabu AO, Atinomo C, Coker AO. High amoxicillin resistance in *Helicobacter pylori* isolated from gastritis and peptic ulcer patients in Western Nigeria. *J. Gastroenterol.* 2001 ; **36** : 67 – 68.
 8. Holcombe C, Kaluba J, Lucas SB. *Helicobacter pylori* infection and gastritis in healthy Nigerians. *Eur. J. Epidemiol.* 1994 ; **10** : 223 – 225.
 9. Bell GD, Powell KU, Burridge SM, et al. *Helicobacter pylori* eradication efficacy and side effect profile of a combination of omeperazole, amoxicillin and metronidazole compared with four alternative regimens. *Q. J. Med. J. Assoc. Phys.* 1993 ; **86** : 743 – 750.
 10. O’Gara EA, Hill DJ, Maslin DJ. Activities of garlic oil, garlic powder and their diallyl constituents against *Helicobacter pylori*. *Appl. Environ. Microbiol.* 2000 ; **66** : 2269 – 2273.
 11. Cavallito CJ, Bouley JH. Allicin, the antibacterial principle of *Allium sativum*. I. Isolation, physical properties and antibacterial action. *J. Am. Chem. Soc.* 1944 ; **66** : 1950-1954.
 12. Cellini L, DiCampli E, Masuli M, DiBartolomeo S, Allocati N. Inhibition of *Helicobacter pylori* by garlic extract (*Allium sativum*). *FEMS. Immunol. Med. Microbiol.* 1996 ; **13** : 273 – 277.

13. Fabry W, Okemo P, Ansorg R. Activity of East African medicinal plants against *Helicobacter pylori*. *Chemother.* 1996 ; **42** : 315 - 317.
14. Makinde JM, Obih PO. Screening of *Morinda lucida* leaf extract for antimalaria action on *Plasmodium berghei berghei* mice. *Afr. J. Med. Med. Sci.* 1985 ; **14** : 59 - 63.
15. Ilori MO, Sheteolu AO, Omonigbehin EA, Adeneye AA. Antidiarrhoeal activities of *Ocimum gratissimum* (*Lamiaceae*). *J. Diarrh. Dis. Res.* 1996 ; **14** : 283- 285.
16. Emeruwa, AC. Antibacterial substance from *Carica papaya* fruit extract. *J. Natur. Products.* 1988 ; **45** : 123 - 127.
17. Sofowora A. The present status of knowledge of the plants used in traditional medicine in West Africa. A medical approach and a chemical evaluation. *J. Ethnopharmacol.* 1980 ; **2** : 109 - 118.
18. Olukoya DK, Idika N, Odugbemi TO. Antibacterial activity of some medicinal plants from Nigeria. *J. Ethnopharmacol.* 1993 ; **36** : 69 - 72.
19. Agomo PU, Idigo JC, Afolabi BM. "Antimalarial" medicinal plants and their impact in cell populations in various organs of mice. *Afr. J. Med. Med. Sci.* 1992 ; **21** : 39 - 46.
20. Offiah VN, Chikwendu UA. Antidiarrhoeal effects of *Ocimum gratissimum* leaf extract in experimental animals. *J. Ethnopharmacol.* 1999 ; **68** : 327 - 330.
21. Angers P, Morales MR, Simon JE. Fatty acid variation in seed oil among *Ocimum* species. *J. Am. Oil. Chem. Soc.* 1996 ; **73** : 393-395.
22. Udoh P, Kehinde A. Studies on antifertility effect of pawpaw seeds (*Carica papaya*) on gonads of male albino rats. *Phytother. Res.* 1999 ; **13** : 226 - 228.