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Original Research Article

EVALUATION OF ANTIBACTERIAL EFFECTS OF *Ficus cycomorus* EXTRACTS ON DRUG RESISTANT *Yersinia enterocolitica* ISOLATES ASSOCIATED WITH APPENDICITIS IN KANO STATE- NIGERIA

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ABSTRACT

The study aimed at evaluating the effects of *Ficus cycomorus* extracts on resistant *Yersinia enterocolitica* isolates associated with appendicitis, processed and unprocessed meat samples in Kano state- Nigeria. Hospitals where appendectomies and laparotomies were carried out was randomly selected for the collection of appendiceal and stool samples from one hundred and ten patients (110) using judgmental sampling technique making total samples two hundred and twenty (220). One hundred and twenty (120) both processed (60) and unprocessed (60) meat samples were randomly collected for processing using culture and biochemical test (API 20E SYSTEM). Antibiogram, phytochemical screening, toxicity, and antibacterial activity of the plant extract on the isolates were also determined. The study revealed that *Yersinia enterocolitica* isolates from appendiceal and stool samples were found sensitive to gentamicin, ciprofloxacin, cifixime, chloramphenicol, and ceftriaxone and resistant to ampicillin while the isolates from unprocessed and processed meat samples were found sensitive to ampicillin, tetracycline, and cifixime and resistant to ceftrixone. The study revealed no significant difference in terms of susceptibility pattern of the isolates to conventional antibiotics (p=0.2). The study revealed insignificant difference (p=0.2) for extract of *Ficus cycomorus* on the isolates and the tolerability of the plant extracts for human use.

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INTRODUCTION

Yersinia enterocolitica is a Gram-negative bacterium that can cause a range of illnesses, from mild gastroenteritis to more severe extra-intestinal manifestations such as appendicitis [14,20]. The increasing prevalence of antibiotic resistance in *Y. enterocolitica* poses a significant challenge to effective treatment [6]. This study investigates the antibiotic resistance profile of *Y. enterocolitica* strains isolated from patients with appendicitis in Kano State, Nigeria, and evaluates the potential of *Ficus cycomorus* plant extracts as alternative antibacterial agents. The rationale for exploring plant extracts stems from the growing concern over antibiotic resistance and the need for novel therapeutic strategies [3]. The selection of *Ficus cycomorus* is based on its traditional use in ethnomedicine for treating infectious diseases,

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suggesting potential antimicrobial properties. This study aims to contribute to a better understanding of *Y. enterocolitica* antibiotic resistance in this specific region and explore the potential of phytotherapy as a complementary approach.

MATERIALS AND METHODS

Appendiceal specimens or surgical resections from laparotomies were collected immediately from each operation and immersed in disposable sterile containers containing peptone physiological saline solution and kept in an ice box (±25 °C) for bacteriological isolation. About 1–2 g of fecal sample was collected, added to a tube containing 10 mL of phosphate-buffered saline, vortexed, homogenized for about 30 s, and incubated at 4 °C for three weeks for bacteriological isolation using Yersinia selective medium cefsulodin–irgasan–novobiocin (CIN) agar [2].

Confirmation of *Yersinia enterocolitica* isolates was done using biochemical testing (API 20E system). Antibiotic susceptibility testing was carried out using the disc diffusion method [13]. Phytochemical screening [7], toxicity testing [9,15], and antibacterial activity of the plant extracts on the isolates were also determined following standard guidelines [5].

RESULTS

The result of the study in (table 1) revealed that *Yersinia enterocolitica* isolates from appendiceal and stool samples were found sensitive to gentamycine, ciprofloxacine, cifixime, chloramphenicol, ceftriazone and resistant to ampicillin. There was no significant difference in terms of susceptibility pattern of all the *Y.enterocolitica* isolates to the conventional antibiotics (P=0.2)

The result in (table 2 and 3) revealed high yield plant extracts upon usage of methanol solvent for *Ficus cycomorus* (6%) The result further revealed detection of bioactive compounds such as saponins, tannins, reducing sugar, glycosides, steroids, phenols, flavonoids and terpenoids. The result also revealed fewer yields in plant extraction with ethyl acetate and N-hexane as solvent and nil protein detected during plant extraction.

The result in (table 4) revealed susceptibility of *Yersinia enterocolitica* isolates to extract of *Ficus cycomorus* at concentration of 200mg/ml and 400mg/ml and resistant at concentration of 50mg/ml and 100mg/ml. There was no significant difference in terms of susceptibility pattern of all the *Y. Enterocolitica* isolates to *F. cycomorus* extract (P=0.2).

The result of biopses revealed unremarkable effect of *Ficus cycomorus* extract on liver, kidney and heart tissue upon acute toxicity test (LD50) indicating tolerability of the plant extracts.

The result in (table 5) also revealed effect of *Ficus cycomorus* extract on hematological parameters fallen within the normal range except at the level of lymphocytes and blood platelets indicating increase in the parameter including the control group of the experimental animals. This may probably indicates presence of viruses as well as reservoir of infection.

The result in (table 6) revealed effect of *Ficus cycomorus* extract on liver function, indicating liver enzymes such as alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphate(ALP) and gamma-glutamyltransferase fallen within normal range indicating tolerability of the extract.

Table 1: Susceptibility of *Y. enterocolitica* Isolates to Conventional Antibiotics

	ANTIBIOTICS					
ISOLATES	AMP	CN	TE	CIP	SXT	C	CRO
Appendiceal	R	S	I	S	S	S	S
Stool	R	S	I	S	S	S	S
Unprocessed meat	S	I	S	I	S	I	R
Processed meat	S	I	S	I	S	I	R

P=0.2

Table 2: Physical Properties of Plant Extract

Extract code	Texture	Color	%yield
EB	Gummy	Greenish	0.5
MB	Crystalline	Reddish brown	6
HB	Gummy	Yellowish green	0.4

KEY: EB = ethyl acetate extract of *Ficus cycomorus*, MB= methanol extract of *Ficus cycomorus*, HB= N-hexane extract of *Ficus cycomorus*.

Table3: Phytochemical constituents of *F. Cycomorus* stem back extract

Phytochemicals	EB	MB	HB
Saponins	+	-	+
Tannins	+	+	+
Reducing sugar	+	+	+
Protein	-	-	-
Glycoside	+	+	+
Steroids	+	+	+
Phenols	+	+	+
Flavonoids	+	+	+
Terpenoids	+	+	+

KEY: EB = ethyl acetate extract of *Ficus cycomorus,* MB= methanol extract of *Ficus cycomorus,* HB=N-hexane extract of *Ficus cycomorus*.

Table 4: Susceptibility of *Y. Enterocolitica* isolates to *F. cycomorus* extracts

	Extract Concentration/Zone Diameter(mm)			
ISOLATES	400mg/ml	200mg/ml	100mg/ml	50mg/ml
Appendiceal	21.6	18.1	14.8	11.1
Stool	21.5	17.5	14.6	12
Unprocessed meat	22.4	18.6	14.7	11.3
Processed meat	23.9	17.7	14.7	11

P= 0.2

KEY: antimicrobial break points (CLSI, 2023)

Susceptible ≥ 20, Intermediate: 15-19 and Resistant: ≤ 14

Table 5: Effects of *F. cycomorus* on hematological parameters

	Extract concentration					
Hematological parameters	0.2g	0.3g	1g	2g	Control	Ref. Range
WBC($X10^9$/L)	7.6	5.77	5.77	6.16	4.78	4-10
LYM(%)	61.6	63.2	60.9	65.4	62.1	20-50
MID(%)	9.8	8.8	10.7	10.9	12.5	3-10
GRA(%)	28.5	25.4	26.1	24.1	23.9	40-70
RBC($X10^{12}$/L)	4.4	4.2	4.2	3.9	4.6	3.8-5.8
HGB(g/dl)	11.7	9.9	10.7	9.5	11.5	11-16.5

Table 1: Susceptibility of *Y. enterocolitica* Isolates to Conventional Antibiotics

	ANTIBIOTICS					
ISOLATES	**AMP**	**CN**	**TE**	**CIP**	**SXT**	**C**	**CRO**
Appendiceal	R	S	I	S	S	S	S
Stool	R	S	I	S	S	S	S
Unprocessed meat	S	I	S	I	S	I	R
Processed meat	S	I	S	I	S	I	R

P=0.2

Table 2: Physical Properties of Plant Extract

Extract code	Texture	Color	%yield
EB	Gummy	Greenish	0.5
MB	Crystalline	Reddish brown	6
HB	Gummy	Yellowish green	0.4

KEY: EB = ethyl acetate extract of *Ficus cycomorus*, MB= methanol extract of *Ficus cycomorus*, HB= N-hexane extract of *Ficus cycomorus*.

Table 3: Phytochemical constituents of *F. Cycomorus* stem back extract

Phytochemicals	EB	MB	HB
Saponins	+	-	+
Tannins	+	+	+
Reducing sugar	+	+	+
Protein	-	-	-
Glycoside	+	+	+
Steroids	+	+	+
Phenols	+	+	+
Flavonoids	+	+	+
Terpenoids	+	+	+

KEY: EB = ethyl acetate extract of *Ficus cycomorus,* MB= methanol extract of *Ficus cycomorus,* HB=N-hexane extract of *Ficus cycomorus*.

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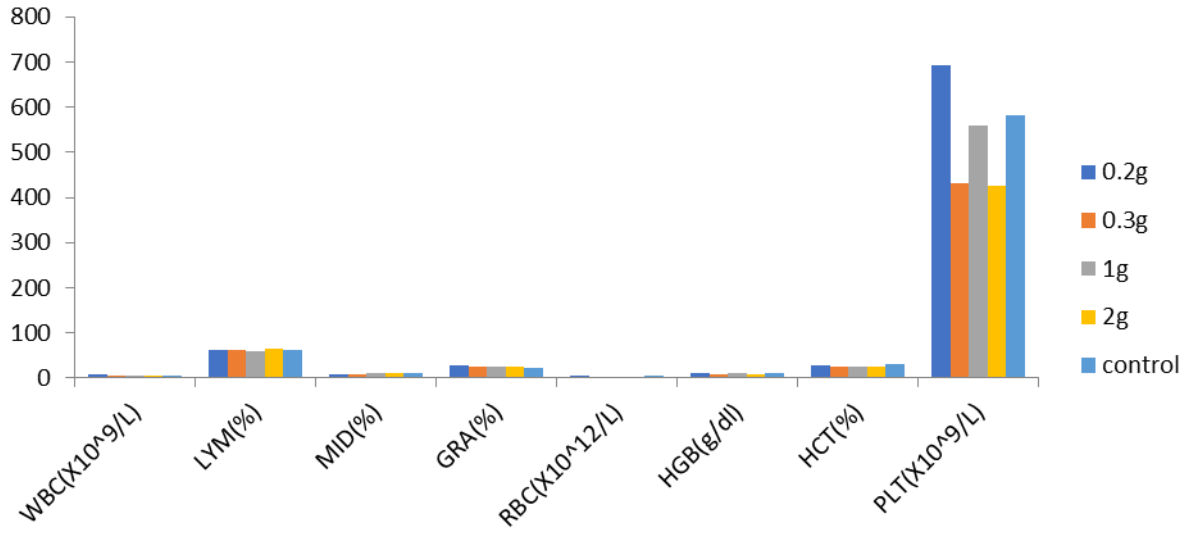
Susceptible ≥ 20, Intermediate: 15-19 and Resistant: ≤ 14

Table 5: Effects of *F. cycomorus* on hematological parameters

	Extract concentration					
Hematological parameters	**0.2g**	**0.3g**	**1g**	**2g**	**Control**	**Ref. Range**
WBC(X10^9/L)	7.6	5.77	5.77	6.16	4.78	4-10
LYM(%)	61.6	63.2	60.9	65.4	62.1	20-50
MID(%)	9.8	8.8	10.7	10.9	12.5	3-10
GRA(%)	28.5	25.4	26.1	24.1	23.9	40-70
RBC(X10^{12}/L)	4.4	4.2	4.2	3.9	4.6	3.8-5.8

HGB(g/dl)	11.7	9.9	10.7	9.5	11.5	11-16.5
HCT(%)	29.5	25.5	24.6	24.1	32.3	35-50
PLT(X10^9/L)	692	432	559	426	581	85-303



P= 0.2

Figure 1: Effects of *F. cycomorus* on hematological parameters

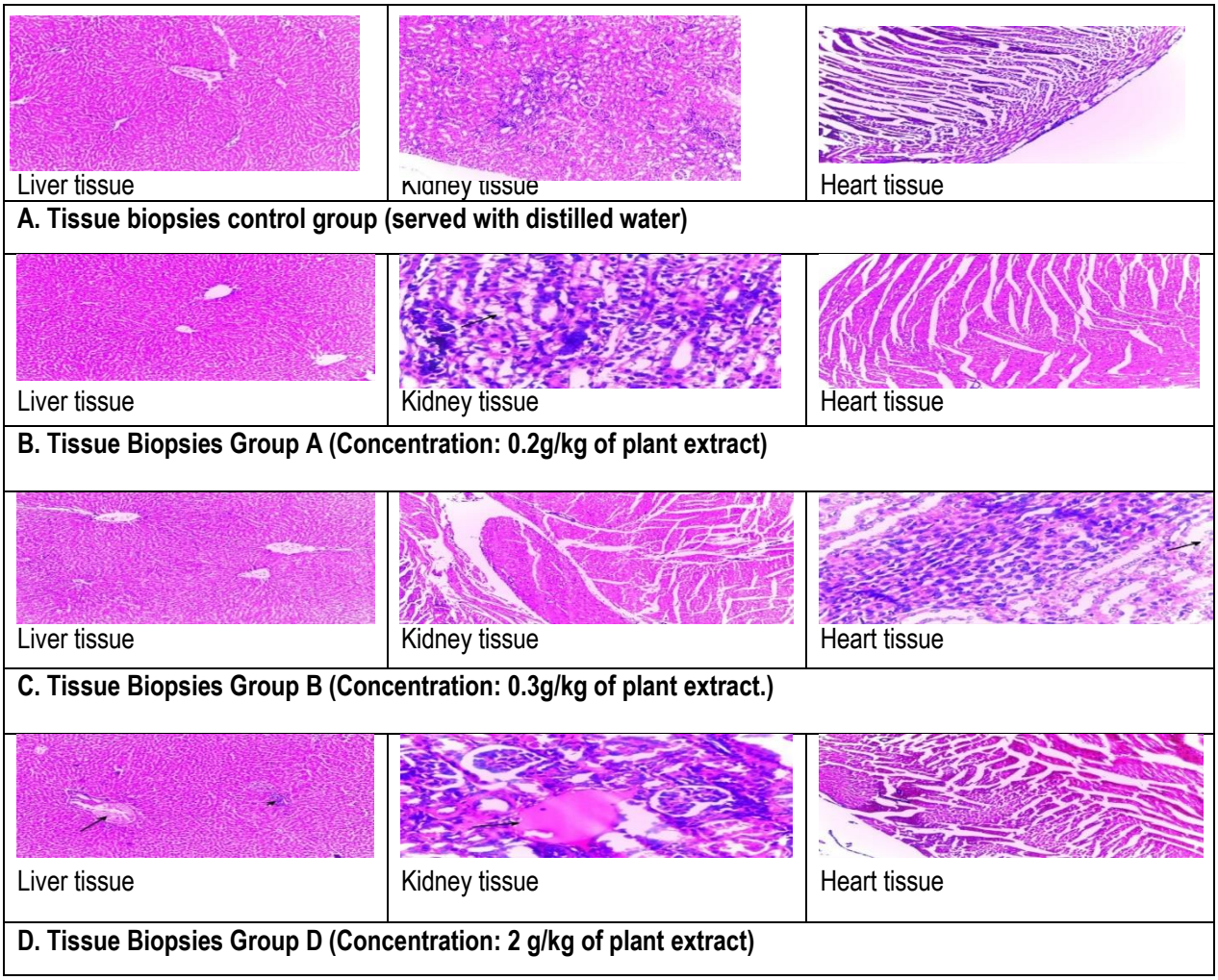


Table 6: Effects of *F. cycomorus* on liver function

Parameters	Extract concentration					
	0.2g	0.3g	1g	2g	Control	Ref. Range
ALT(IU/L)	33	34.2	35.7	31.5	33.5	0-45
AST(IU/L)	25	27.5	30.4	33.3	28.2	0-35
ALP(IU/L)	40	43.4	39.3	41.4	39.4	30-120
GGT(IU/L)	20	24.3	22.5	25.2	23	0-30

P = 0.2

DISCUSSION

This study highlights the concerning prevalence of antibiotic resistance among *Y. enterocolitica* strains associated with appendicitis in Kano State. The findings are consistent with other studies reporting high levels of antibiotic resistance in *Y. enterocolitica* from various sources [14,18].

Antimicrobial susceptibility testing revealed resistance to ampicillin, a finding expected due to the intrinsic β-lactamase activity of *Y. enterocolitica*. However, clinical isolates retained susceptibility to gentamicin, ciprofloxacin, and ceftriaxone, supporting the continued efficacy of these agents in treatment [11]. These findings underscore the importance of ongoing surveillance to monitor emerging resistance trends in both clinical and environmental settings.

The study also revealed resistance to ampicillin (100%) and susceptibility to ciprofloxacin (92%) and gentamicin (89%), reflecting global resistance patterns. Ampicillin resistance is attributed to intrinsic β-lactamase activity [8], while ciprofloxacin remains effective due to restricted veterinary use in Nigeria. These findings emphasize the need for antibiotic stewardship to curb multidrug resistance, a looming threat highlighted by the World Health Organization [20].

Evaluation of plant-derived antimicrobials demonstrated that *Ficus cycomorus* extracts exhibited dose-dependent inhibitory effects against *Y. enterocolitica*. suggesting a higher concentration or enhanced efficacy of bioactive compounds. This aligns with reports on natural antimicrobials, including marine algal extracts shown to inhibit *Y. enterocolitica* in dairy products [12].

The superior efficacy of *F. cycomorus* (23.9 mm) may be due to higher concentrations of tannins and flavonoids, which disrupt bacterial membranes [19]. Similar dose-dependent activity has been reported in *Ficus* species against enteropathogens [16].

Toxicological assessments revealed that the extract was generally well tolerated, with normal hematological and liver function parameters. Acute toxicity tests (LD_{50} > 5000 mg/kg) further confirmed safety, consistent with previous findings [4]. Mild hematological shifts may reflect immunomodulatory effects, as *Ficus* species are rich in saponins and alkaloids [10].

From a public health perspective, this study highlights *Y. enterocolitica* as a potential contributor to appendicitis misdiagnosis in Nigeria, consistent with previous observations [1]. The efficacy of *Ficus* extracts offers a promising alternative amid rising antimicrobial resistance, particularly in rural settings reliant on traditional medicine [17].

CONCLUSION

This study demonstrates a high prevalence of antibiotic resistance in *Y.enterocolitica* strains isolated from patients with appendicitis in Kano State. The significant antibacterial activity of *Ficus cycomorus* extracts offers promise for developing novel therapeutic strategies to combat antibiotic-resistant *Y. enterocolitica*. Further research, including larger-scale studies and preclinical/clinical trials, is warranted to validate these findings and explore the potential of plant-based therapies in managing *Y. enterocolitica* infections. This research underscores the critical need for continuous surveillance of antibiotic resistance patterns in *Y. enterocolitica* and the exploration of alternative treatment modalities to address this growing public health concern.

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AUTHORS' CONTRIBUTION

YSM and AAI designed the work. YSM carried out the research under the supervision of AAI. YSM drafted the original manuscript. YSM, AAI, and MMD revised the manuscript and provided technical guidance. All authors read, made comments, and agreed on the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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