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Synergy and Antagonism in Polyherbal Combinations of Selected Zimbabwean Medicinal Plant Extracts Against *Neisseria gonorrhoeae*

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Abstract

Neisseria gonorrhoeae has rendered several antibiotic classes ineffective as it continues to acquire resistance. This has presented an urgent need for alternative therapeutic strategies, and herbal medicines have shown great potential. This study aims to evaluate the interaction outcomes of five medicinal plant extracts, used alone and in combination, against the ATCC 12700 *strain of Neisseria gonorrhoeae*. Fine powders of *Azadirachta indica*, *Hypoxis hemerocallidea*, *Erythrina abyssinica*, *Kigelia africana,* and *Xeroderris stuhlmannii* were subjected to cold maceration using ethanol as the solvent. The antimicrobial potential of individual and combined herbal extracts was tested on Thayer-Martin agar using the disc diffusion and agar-well diffusion methods. Furthermore, a fixed-ratio checkerboard assay and interaction profiles were determined using Minimum Inhibitory Concentration (MIC) values and the Fractional Inhibitory Concentration Index (FICI), and the results were interpreted as synergy,

antagonism, indifference, or additivity. Among the single extracts, *H. hemerocallidea* showed the highest potency (MIC 5.44 mg/mL), whereas *A. indica* demonstrated the weakest activity (MIC 100 mg/mL). Strong synergy was detected for *A. indica* + *E. abyssinica* (FICI 0.24), *K. africana* + *X. stuhlmannii* (FICI 0.30), and *K. africana* + *E. abyssinica* (FICI 0.40), while the triple combination *A. indica* + *K. africana* + *E. abyssinica* produced the strongest synergistic effect overall (FICI 0.10). In contrast, several mixtures were antagonistic, including *A. indica* + *K. africana* (FICI 9.69), indicating that increasing formulation complexity did not consistently translate into improved anti-gonococcal efficacy. The most potent combination was subjected to Gas Chromatography-Mass Spectrometry (GC-MS) to identify phytochemical compounds. GC-MS identified terpenoids, phenols, steroids, and alkaloids. Overall, the findings show that increasing the number of extracts in a combination does not guarantee synergistic outcomes; hence, the need for ratio optimization and mechanism-guided combination design in follow-up studies.

Keywords: *Neisseria gonorrhoeae, medicinal plant extracts, polyherbal combinations, synergy, fractional inhibitory concentration index, antimicrobial resistance.*

Introduction

Gonorrhoea is a sexually transmitted infection caused by *Neisseria gonorrhoeae*, a Gram-negative diplococcal bacterium [1]. The World Health Organization estimates that approximately 82.4 million new gonorrhea infections occur globally each year, equivalent to more than 200,000 infections daily [2]. Gonorrhoea remains one of the most frequently reported bacterial sexually transmitted infections worldwide, with a substantial burden in sub-Saharan Africa, particularly among adolescents and young adults [3,4]. *Neisseria gonorrhoeae* has been designated a priority pathogen for the research and development of alternative treatments because of rapidly evolving resistance, including reduced susceptibility to ceftriaxone [5,6]. Consequently, there is an urgent need to identify novel anti-gonococcal agents. In Zimbabwe, the public health relevance of gonorrhea and related STI control is reinforced by recent evidence of a high burden of curable STIs in key populations and by the implementation of the WHO Enhanced Gonococcal Antimicrobial Surveillance Program to strengthen national monitoring of gonococcal antimicrobial resistance [34,35].

Herbal medicines have been used for centuries [7], and recent studies have reinforced their potential as sources of antimicrobial agents against multidrug-resistant pathogens [8]. Medicinal plants such as *Kigelia africana*, *Azadirachta indica*, *Hypoxis hemerocallidea*, *Xeroderris stuhlmannii,* and

Erythrina abyssinica are widely distributed in southern Africa and have demonstrated activity against Gram-negative bacteria, suggesting possible inhibitory effects on *Neisseria gonorrhoeae* [9]. In African traditional medicine, polyherbal formulations are commonly used with the expectation that combining plants may result in synergy that enhances efficacy, lowers the required dose, and broadens therapeutic activity [10].

Synergy occurs when the combined effect of bioactive compounds exceeds the sum of their individual effects [11]. However, multicomponent herbal mixtures may also produce additive, indifferent, or antagonistic interactions [12]. Scientific evidence validating synergistic effects in polyherbal preparations remains limited, and most published studies have focused on single-plant extracts rather than systematically optimized combinations. Given the ongoing rise in antimicrobial resistance among pathogens such as *Neisseria gonorrhoeae*, it is important to assess both the activity of individual medicinal plant extracts and the interaction profiles of their combinations.

This study evaluated the anti-gonococcal activity of selected medicinal plant extracts and determined whether combining extracts improved activity against *Neisseria gonorrhoeae*. Specifically, the study assessed the antibacterial activity of individual extracts using their estimated minimum inhibitory concentration values and examined the interaction effects of paired, triple, quadruple, and five-extract combinations using checkerboard-derived fractional inhibitory concentration index analysis.

Materials and Methods

Plant collection and identification

Fresh leaves of *Azadirachta indica*, corms of *Hypoxis hemerocallidea*, and bark from *Erythrina abyssinica*, *Kigelia africana,* and *Xeroderris stuhlmannii* were collected in (-17.74138,30.89676) Harare, Zimbabwe. Plant identity was confirmed by a botanist, and voucher specimens FPC-NYB-072/AI, FPC-NYB-073/EA, FPC-NYB-074/XS, FPC-NYB-076/KA, and FPC-NYB-077/HH were assigned and deposited at the Fambidzanai Permaculture Center herbarium.

Preparation of extracts

Plant materials were washed with distilled water, air-dried for 10 days in the shade away from direct sunlight, and then ground into fine powder using an industrial blender. Fifty grams of each powdered sample was

macerated in 150 mL of 95% ethanol at room temperature for 72 hours in a shaking incubator. After maceration, the mixture was filtered using Whatman No. 1 filter paper and concentrated to dryness in a water bath at 60 °C [13,14]. The crude extracts were weighed using an analytical balance, and the percentage yield was calculated.

Yield (%) = (crude extract obtained / initial weight) x 100

The crude extracts were stored in airtight containers at 4°C until use.

Phytochemical Analysis

Phytochemical screening was conducted to detect the presence of key phytochemical classes, namely: tannins, saponins, flavonoids, phenols, alkaloids, glycosides, steroids, and terpenoids, following the method without modification [13].

Test for Tannins

Approximately 0.5 g of crude plant extract was boiled with 5 mL of distilled water for 2 minutes and allowed to cool. Three drops of 0.1% ferric chloride ($FeCl_3$) were then added. The appearance of a blue-green precipitate indicated the presence of tannins.

Test for Alkaloids

Two milliliters of plant extract were mixed with 1 mL of Mayer-Wagner reagent. The formation of a pale yellow or cream-colored precipitate confirmed the presence of alkaloids.

Test for Cardiac Glycosides

Crude plant extract (5 mL) was mixed with 2 mL of concentrated glacial acetic acid, followed by one drop of 10% ferric chloride. The appearance of a greenish or violet ring at the interface indicated the presence of cardiac glycosides.

Test for Saponins

Powdered plant extract (0.5 g) was diluted with 2 mL of distilled water in a test tube and shaken vigorously for 15 minutes. The formation of a stable froth indicated the presence of saponins.

Test for Steroids

Crude plant extract (2 mL) was mixed with 2 mL of chloroform, after which concentrated sulfuric acid was carefully added down the side of the test tube. Formation of a reddish-brown ring at the interface indicated the presence of steroids.

Test for Terpenoids

Five milliliters of plant extract were dissolved in 2 mL of chloroform; 3 mL of concentrated sulfuric acid was then added gently along the side of

the test tube. A reddish-brown coloration indicated the presence of terpenoids.

Test for Phenols

About 0.5 g of crude extract was placed in a test tube, and 5 mL of 5% ferric chloride ($FeCl_3$) was added. The development of a dark green or bluish-black color indicated the presence of phenols.

Test for Flavonoids

Approximately 1 mL of crude extract was mixed with a few drops of 2% sodium hydroxide solution. The formation of a yellow color that became colorless after the addition of dilute hydrochloric acid indicated the presence of flavonoids.

Culture conditions and confirmation of N. gonorrhoeae isolates.

Neisseria gonorrhoeae strains were obtained from the National Microbiology Reference Laboratory at Sally Mugabe Central Hospital, Zimbabwe. The isolates were sub-cultured on Thayer-Martin agar supplemented with 5% sheep blood and vancomycin, colistin, nystatin, and trimethoprim (VCNT) to suppress contaminating microorganisms. Plates were inoculated using a sterile cotton swab and incubated at 37 °C in a 5% carbon dioxide incubator for 32 hours. Colony morphology and Gram staining confirmed Gram-negative, kidney-shaped diplococci consistent with *Neisseria gonorrhoeae*.

Antimicrobial screening of extracts

The ethanolic extracts of *Azadirachta indica*, *Hypoxis hemerocallidea*, *Erythrina abyssinica*, *Kigelia africana,* and *Xeroderris stuhlmannii* were used to prepare a 200 mg/mL stock solution in ethanol. Serial two-fold dilutions were then prepared to obtain concentrations of 100, 50, 25, and 12.5 mg/mL. Filter paper discs (8 mm) were impregnated with 20 µL of each concentration and air-dried at room temperature for 30 minutes to allow solvent evaporation. The bacterial suspension was prepared by transferring colonies into 4 mL of sterile saline (0.85% NaCl) until the turbidity matched a 0.5 McFarland standard. Freshly prepared Thayer-Martin agar plates were inoculated by evenly spreading the bacterial suspension with a swab. The extract-impregnated discs were gently pressed onto the agar using flame-sterilized forceps to ensure full contact. Ceftriaxone (100 mg/mL) and pure ethanol were used as the positive and negative controls, respectively. The plates were left at room temperature for 30 minutes to allow pre-incubation diffusion, then incubated at 37 °C in a 5% CO2 incubator for 32 hours. All tests were

performed in triplicate, and zones of inhibition were measured and recorded.

Determination of Minimum Inhibitory Concentration of extracts

After recording the zones of inhibition, the mean radius was calculated using the following formula:

Mean radius (mm) = (Mean diameter – Disc diameter (8mm))/2

To estimate the minimum inhibitory concentration (MIC) of each extract, the square of the inhibition radius was plotted against the base-10 logarithm of extract concentration in Microsoft Excel. The x-intercept of the line of best fit was taken as the point from which the estimated MIC was derived, and the antilog of the x-intercept was reported as the concentration at which visible bacterial growth was completely inhibited.

Combinational studies

A modified agar-based fixed-ratio checkerboard approach was used to evaluate the interaction effects of different extract combinations [15,16]. Stock solutions were prepared from the predetermined MIC values, and each extract was adjusted to 4x MIC before mixing with the companion extract or extracts at a 1:1 ratio for paired, triple, quadruple, and five-extract combinations. Two-fold serial dilutions were then prepared to obtain five concentrations: 4× MIC, 2× MIC, 1× MIC, MIC/2, and MIC/4. Uniform wells (8 mm) were bored into freshly prepared Thayer-Martin agar plates previously inoculated with *Neisseria gonorrhoeae*. One hundred microliters of each combination were dispensed into the wells using a calibrated micropipette, and the plates were incubated at 37 °C under 3-5% CO2 for 32 hours. Each assay was performed in triplicate, and zones of inhibition were measured in millimeters for analysis.

Determination of the MIC of the combination extracts

The mean diameter of the zones of inhibition from triplicate measurements was calculated using the formula:

Mean diameter (mm) = (D1 + D2 + D3)/ 3

The mean inhibition radius was calculated using the formula:

Mean radius (mm) = (Mean diameter – Disc diameter (8mm))/2

The square of the mean radius was plotted against the logarithm of extract concentration using Microsoft Excel (version 2019). A regression

line of best fit was generated, and the x-intercept of the most linear portion was used to estimate the MIC. The actual value was obtained by calculating the antilogarithm of the x-intercept.

Fractional inhibitory concentration index and interpretation analysis

The Fractional inhibitory concentration index was calculated using the formula [16]:

FICI = ΣFIC = FIC (plant extract 1) + FIC (plant extract 2) + FIC (plant extract 3) + FIC (plant extract 4) + FIC (plant extract 5)

Where:

FIC (extract 1) = MIC of extract 1 in combination / MIC of 1 extract alone.

The FICI was interpreted using the following criteria.

FICI range	Interaction type
≤ 0.5	Synergistic
0.5 – 1.0	Additive
>1.0 – ≤4.0	Indifferent
>4.0	Antagonistic

Chromatography-Mass Spectrometry (GC-MS) Analysis.

For further phytochemical characterization, a combined sample containing *Azadirachta indica*, *Erythrina abyssinica,* and *Kigelia africana* was analyzed by gas chromatography-mass spectrometry (GC-MS) at the Environmental Management Agency laboratory in Harare, Zimbabwe. Compounds were identified by comparing spectra with entries in the National Institute of Standards and Technology (NIST) library, and matches greater than 80% similarity were accepted.

Statistical analysis

Quantitative data from the antimicrobial assays of individual extracts and extract combinations were analyzed using one-way analysis of variance (ANOVA) to compare mean zones of inhibition across treatment groups. Tukey's honest significant difference test was used for post hoc comparisons where appropriate, and statistical significance was set at $p <= 0.05$. Because the primary objective of the study was comparative screening, the results are interpreted as relative differences in activity among treatments.

Results

All reported inhibition data were generated from triplicate assays, and the tables below present the descriptive outcomes of extraction yield, phytochemical screening, control performance, estimated MIC values, and extract-combination interactions.

Table 1. Percentage yield of crude ethanolic extracts from selected medicinal plants

Plant	Percentage yield (%)
A. indica	7.6
E. abyssinica	8.2
K. africana	3.0
H. hemerocallidea	5.6
X. stuhlmannii	6.2

Table 1 shows the extraction yield obtained from each medicinal plant after ethanolic maceration and solvent removal. Variation in yield was observed, with *K. africana* showing the lowest percentage yield.

Table 2. Qualitative phytochemical profile of the selected medicinal plant extracts

	Phytochemical							
Plant	Steroids	Terpenoids	Phenols	Tannins	Flavonoids	Alkaloids	Glycoside s	Saponins
A. indica	+	-	+	+	+	+	+	+
E. abyssinica	-	+	+	+	+	+	+	+
K. africana	-	+	+	+	-	+	-	+
H. Hemerocallidea	+	+	-	-	-	-	-	-
X. stuhlmannii	-	+	-	+	+	-	+	-

Table 2 summarizes the qualitative phytochemical screening results for the selected plant extracts. *A. indica* and *E. abyssinica* had the most classes of phytochemical constituents detected, and *H. hemerocallidea* had the least.

Table 3. Mean zones of inhibition produced by positive and negative controls

Control	Mean diameter of Zones (mm)
Positive control (Ceftriaxone 100 mg/mL)	38.00
Negative control (Ethanol 95%)	0.00

Table 3 presents the inhibition zones recorded for the positive and negative controls used during antimicrobial testing.

Table 4. X-intercepts and estimated minimum inhibitory concentrations (MICs) of individual extracts

Extract	X-Intercept	MIC (mg/mL)
A. indica	2	100.00
E. abyssinica	1.44	27.60
H. hemerocallidea	0.74	5.44
X. stuhlmannii	1.57	36.99
K. africana	1.51	32.56

Table 4 presents the estimated MIC values derived from the x-intercepts of the dose–response plots for the individual extracts. *A. indica* has the greatest MIC value of 100 mg/ml, while *H. hemerocallidea* has the least MIC value of 5.44 mg/ml.

Table 5. Fractional inhibitory concentration indices (FICI) and interaction outcomes of extract combinations

Combination	Extract	MIC in Combination (mg/mL)	MIC Alone (mg/mL)	FIC	FICI	Type of Interaction
H+K	**H**	3.54	5.44	0.65		
	K	21.17	32.56	0.65	1.30	**Indifferent**
H+E	**H**	4.04	5.44	0.75		
	E	20.80	27.60	0.74	1.50	**Indifferent**
A+K	**A**	489.32	100.00	4.89		
	K	156.32	32.56	4.80	9.69	**Antagonistic**
A+E	**A**	11.88	100.00	0.12		
	E	3.28	27.60	0.12	0.24	**Synergistic**
K+X	**K**	4.88	32.56	0.15		
	X	5.55	36.99	0.15	0.30	**Synergistic**
K+E	**K**	6.51	32.56	0.20		
	E	5.52	27.60	0.20	0.40	**Synergistic**
H+A+K	**H**	0.55	5.44	0.10		
	A	0.55	100.00	0.01		
	K	3.29	32.56	0.10	0.21	**Synergistic**

H+A+X	**H**	10.57	5.44	1.94		
	A	194.31	100.00	1.94		
	X	71.87	36.99	1.94	5.83	**Antagonistic**
H+K+E	**H**	1.32	5.44	0.24		
	K	5.56	32.56	0.17		
	E	3.28	27.60	0.12	0.54	**Additive**
H+X+E	**H**	9.31	5.44	1.71		
	X	63.30	36.99	1.71		
	E	47.23	27.60	1.71	5.13	**Antagonistic**
A+K+E	**A**	3.33	100.00	0.01		
	K	1.09	32.56	0.03		
	E	0.90	27.60	0.03	0.10	**Synergistic**
H+A+X+E	**H**	5.92	5.44	1.09		
	A	97.15	100.00	0.97		
	X	35.94	36.99	0.97		
	E	27.20	27.60	0.99	4.02	**Antagonistic**
H+K+X+E	**H**	3.99	5.44	0.62		
	K	9.50	32.56	0.29		
	X	10.80	36.99	0.29		
	E	8.05	27.60	0.29	1.50	**Antagonistic**
H+A+K+X+E	**H**	3.53	5.44	0.65		
	A	64.81	100.00	0.65		
	K	21.10	32.56	0.65		
	X	69.56	36.99	1.88		
	E	18.50	27.60	0.67	4.50	**Antagonistic**

Key: A - *Azadirachta indica*, E - *Erythrina abyssinica*, H - *Hypoxis hemerocallidea*, X - *Xeroderris stuhlmannii,* and K - *Kigelia africana*.

Table 5 summarizes the MICs in combination, the derived FIC/FICI values, and the resulting interaction categories for the tested extract mixtures. It was observed that combinations of more than 3 extracts yielded more antagonistic outcomes.

Figure 1. GC-MS Chromatogram of the A+K+E herbal extract combination.

Significant metabolite peaks were observed from 35 min onwards.

Table 6. GC-MS-identified phytochemicals in the synergistic *A. indica + K. africana + E. abyssinica* combination

	RT (min)	Class and Compound name	Peak area	Match score	Formula
		Terpenoids			
1	36.1	Neophytadiene	3624134	97.5	$C_{20}H_{38}$
2	42.634	Phytol	555894	91.9	$C_{20}H_{40}O$
3	43.843	Octadecanoic acid	1122411	81	$C_{18}H_{36}O_2$
4	39.234	n-Hexadecanoic acid	14327843	95.4	$C_{16}H_{32}O_2$
5	47.733	4,8,12,16-Tetramethylheptadecan-4-olide	386384	82.2	$C_{21}H_{40}O_2$
6	36.281	2-Hexadecene, 3,7,11,15-tetramethyl-	560444	87.1	$C_{20}H_{40}$
7	37.173	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	1078910	94.8	$C_{20}H_{40}O$
8	33.291	1-Hexadecanol, 3,7,11,15-tetramethyl-	456324	91.9	$C_{20}H_{42}O$
9	26.902	Cydoheptasiloxane, tetradecamethyl-	116592	86.2	$C_{14}H_{42}O_7Si_7$
10	21.503	Cydohexasiloxane, dodecamethyl-	61650	83	$C_{12}H_{36}O_6Si_6$
11	64.992	(-)-10-epi-α-Cyperone	205854	80.4	$C_{15}H_{22}O$
12	65.527	Olean-12-en-3-ol (β-amyrin	194352	86	$C_{30}H_{50}O$
		Steroids			
13	64.918	Stigmast-5-en-3-ol	12909961	92.4	$C_{29}H_{48}$
14	63.708	(22E)-Stigmasta-5,22-dien-3-ol	1983625	88	$C_{29}H_{48}O$
15	63.066	(3-Methyl,24R)-ergost-5-en-3-ol	1779681	87.3	$C_{28}H_{48}O$
16	60.79	Stigmastan-3,5-diene	6801808	92.4	$C_{29}H_{48}$
		Alkaloids			
17	12.592	Benzenamine, 4-methyl-	546796	85.2	C_7H_9N
18	12.547	Benzenemethanamine, α-(2-methylpropyl-3-13C)-	177565	89	$C_{11}H_{17}N$
19	12.568	(S)-1-(2-Pyridyl)-2-methylpropylamine	366592	89.3	$C_9H_{14}N_2$
20	21.108	1-(2'-Benzo [1,3] dioxol-5-ylethyl)-1-azaspiro [4.5] dec-6-ene	149602	86.6	$C_{18}H_{23}NO_2$
21	42.741	N-tert-Butyl-N-methyl-2-formyl-1-naphthamide	130841	90.7	$C_{17}H_{19}NO_2$
		Phenols			
22	20.508	Phenol, 2-(1-phenylethyl)-	130399	83.7	$C_{14}H_{14}O$

23	27.203	1,4-Benzenediol, 2-(1,1-dimethylethyl)-5-(2-propenyl)-	175805	88.3	$C_{13}H_{18}O_2$
24	60.261	β-Tocopherol	560403	84.2	$C_{28}H_{48}O_2$
25	61.563	dl-α-Tocopherol	5004454	96.7	$C_{29}H_{50}O_2$
26	28.966	2,6-Dimethyl-8,9-dioxo-2,3,5,6-tetraazabicydo [5.2.0] nona-1(7),3-diene	84976	82.9	$C_7H_8N_4O_2$

Table 6 lists the phytochemical constituents detected by GC-MS in the most synergistic herbal combination. The results identified phytochemical compounds such as terpenoids, steroids, alkaloids, and phenolics.

Discussion

The present study evaluated the antibacterial activity of five selected medicinal plant extracts individually and in polyherbal combinations against *Neisseria gonorrhoeae*. Clear variation was observed among the extracts and among the different combinations, indicating that the interaction outcome depended strongly on formulation composition. Among the individual extracts, *Hypoxis hemerocallidea* yielded the lowest estimated MIC (5.44 mg/mL), whereas *Azadirachta indica* yielded the highest (100 mg/mL), suggesting comparatively weaker activity under the assay conditions. Although *Azadirachta indica* is widely reported to possess broad-spectrum antimicrobial properties [17], it exhibited the highest estimated MIC in the present assay. A plausible explanation is the hydrophobic character of major neem constituents, including limonoids and related triterpenoids [18], which may diffuse poorly through agar and may also cross the outer membrane of Gram-negative bacteria less efficiently [19]. Accordingly, the weaker apparent activity observed here should be interpreted cautiously and in relation to the agar-based screening format used.

In contrast, the comparatively strong activity observed for *Hypoxis hemerocallidea* may be associated with compounds such as sterols, sterolins, norlignan, daucosterol, and rooperol [20]. GC-MS results of the most potent combination also show the presence of steroids. Rooperol, a metabolite derived from hypoxoside, has been linked to membrane disruption and interference with nucleic acid synthesis [21]. This interpretation is consistent with previous reports describing antimicrobial activity of *Hypoxis hemerocallidea* against organisms including *Shigella flexneri*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* [22].

Polyherbal preparations are often used in traditional medicine with the assumption that combining plants enhances therapeutic efficacy [10]. In the present study, interaction outcomes varied substantially and included synergistic, additive, indifferent, and antagonistic effects, as determined by the fractional inhibitory concentration index. The combination of *Azadirachta indica*, *Kigelia africana,* and *Erythrina abyssinica* produced the lowest FICI value (0.10), indicating the strongest synergistic interaction observed. This finding suggests that rationally selected low-order combinations may improve anti-gonococcal activity more effectively than empirically mixed combinations of multiple herbs.

The observed synergy may reflect complementary mechanisms among phytochemical classes present in the extracts. For example, fatty acids such as n-Hexadecanoic acid may affect membrane integrity [26], thereby facilitating the action of other compounds, while alkaloids and phenolics may interfere with intracellular targets and oxidative balance [27]. By acting simultaneously on multiple bacterial processes, these compounds may produce a combined inhibitory effect greater than that of the individual extracts alone.

By contrast, some combinations showed clear antagonism. The pairing of *Azadirachta indica* and *Kigelia africana* produced a high FICI value (9.69), indicating markedly reduced activity in combination relative to the individual extracts. Antagonism in polyherbal mixtures may arise from phytochemical incompatibility, competition among compounds for similar targets, reduced diffusion, or chemical interactions that diminish the activity of one or more constituents [11].

Combinations containing four or five extracts were predominantly antagonistic, and some pairwise combinations involving *Hypoxis hemerocallidea* with *Kigelia africana* or *Erythrina abyssinica* were indifferent. These findings reinforce the point that polyherbal use does not automatically confer synergistic benefit and that increasing formulation complexity may reduce, rather than improve, antibacterial activity. In the wider African setting, where traditional medicine remains a major component of primary health care for much of the population, these findings support the need to move from empirical polyherbal use toward evidence-based optimization, standardization, and safety validation of combinations intended for priority pathogens such as *Neisseria gonorrhoeae* [36].

The therapeutic potential of herbal preparations is influenced by their phytochemical composition, which shapes antimicrobial activity and, in vivo, may also affect host responses [30,31]. In the present study, GC-

MS analysis of the synergistic *Azadirachta indica-Erythrina abyssinica-Kigelia africana* combination, as shown in Table 6, identified terpenoids, steroids, alkaloids, and phenolic compounds, supporting the interpretation that multiple constituent classes may contribute to the observed interaction profile.

The inclusion of qualitative phytochemical screening for the individual extracts and GC-MS profiling for the most synergistic combination, therefore, adds chemical context to the biological findings and improves the interpretability of the observed interaction patterns.

An important limitation of this work is that MIC values were estimated from an agar-based diffusion approach rather than determined by standard broth microdilution methods. The findings should therefore be interpreted primarily as comparative screening evidence. Nevertheless, the results provide a useful basis for selecting promising combinations for follow-up studies using standardized susceptibility assays, phytochemical fractionation, and mechanistic evaluation.

Conclusion

This study supports the antibacterial potential of selected Zimbabwean medicinal plant extracts against *Neisseria gonorrhoeae* under the assay conditions used. *Hypoxis hemerocallidea* showed the strongest activity among the individual extracts, whereas the combination of *Azadirachta indica*, *Erythrina abyssinica,* and *Kigelia africana* produced the strongest synergistic interaction. The findings also show that combining multiple extracts does not necessarily improve efficacy, because additive, indifferent, and antagonistic interactions were also observed. Overall, the results support the need for rational selection and optimization of polyherbal combinations rather than indiscriminate blending.

Recommendations

This study has demonstrated antimicrobial activity among the selected medicinal plant extracts; however, further work is required. Future studies should test resistant *Neisseria gonorrhoeae* strains using standard broth-based susceptibility methods, optimize extract ratios using experimental designs such as Box-Behnken or central composite designs, and investigate phytochemical fractions, pharmacokinetics, and pharmacodynamics. Additional in silico, ex vivo, and in vivo studies are also recommended before clinical translation is considered. Future reports should also present measures of variability, such as standard

deviation or standard error, alongside exact p-values and confidence intervals to strengthen statistical transparency.

Ethical statement:

Ethics approval was not required for this study.

Author contributions:

All authors contributed to the conceptualization, primary investigation, formal analysis, data interpretation, and manuscript writing. All authors have read and agreed to submit the final version of the manuscript.

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Abbreviations

MIC - minimum inhibitory concentration
STI - sexually transmitted infection
WHO - World Health Organization

Declaration of conflict of interest.

All authors confirm that they have no interest to declare

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