Synergistic antimicrobial activity of ethanolic extracts of *Carica papaya* and *Senna didymobotrya* against selected human pathogens in Western Kenya

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ABSTRACT

With the emergence of antimicrobial resistance, efforts have been made to find alternative therapeutic agents, especially medicinal plants. The present study assessed the synergistic antimicrobial effect of ethanol extracts of *Carica papaya* and *Senna didymobotrya* against the microorganisms *Streptococcus pyogenes*, *Staphylococcus aureus*, and *Candida albicans*. Seeds were obtained from four sub-counties of Bungoma County, Kenya, extracted using 95% ethanol, and tested both singly and in combination using the agar well diffusion method. Minimum inhibitory concentrations (MICs) and zones of inhibition (ZOI) were measured. Both extracts were found to contain flavonoids, tannins, saponins, alkaloids, glycosides, phenols, and terpenoids, as revealed by phytochemical screening. Both plants were found to be antimicrobial at concentrations ≥50%. Interestingly, synergistic combinations showed increased antibacterial activity against *S. pyogenes* and *S. aureus*, especially at 50–100% concentrations, but no synergistic activity was seen against *C. albicans*. Based on these results, it can be concluded that the combination of extracts from *C. papaya* and *S. didymobotrya* has potential as an alternative antimicrobial agent with good antibacterial synergy. The study recommends that a scientifically validated combination of *C. papaya* and *S. didymobotrya* ethanolic extracts be included in national antimicrobial resistance strategies and community-level health-resilience programmes as a complementary, locally available intervention to decrease the use of conventional antibiotics, especially in resource-limited settings. To facilitate safe clinical application, further translational research is needed, such as toxicity profiling, dosage standardization, and in vivo validation.

Key words: Synergy, medicinal plants, antimicrobial resistance, Carica papaya, Senna didymobotrya

I. INTRODUCTION

Antimicrobial resistance (AMR) is one of the biggest public health challenges of the 21st century and threatens decades of gains in the control of infectious diseases. AMR is a major problem and a disproportionate burden in low and middle-income countries (LMICs) where the burden of infectious disease is high, and access to effective and affordable antibiotics is limited (World Health Organisation [WHO], 2023). These environments have been associated with the emergence and dissemination of multidrug resistant microbial strains, as a result of the inappropriate and excessive use of broad-spectrum antibiotics, often without proper prescription or diagnostic guidance (Prestinaci *et al*., 2015; O'Neill, 2016).

Staphylococcus aureus, Streptococcus pyogenes and Candida albicans are among the pathogens of major clinical concern. Staphylococcus aureus is a major cause of skin and soft tissue infections, septicemia, pneumonia and endocarditis, and methicillin-resistant S. aureus (MRSA) is a major therapeutic challenge worldwide (Tong *et al*., 2015). Group A streptococcus (GAS) Streptococcus pyogenes causes a variety of diseases including pharyngitis, impetigo, and life-threatening invasive infections like necrotizing fasciitis and streptococcal toxic shock syndrome (Cunningham, 2000). Candida albicans is an opportunistic fungal pathogen, the leading cause of mucosal and systemic candidiasis, especially in immunocompromised patients, and is becoming more resistant to commonly used antifungal drugs like azoles (Pappas *et al*., 2016).

The lack of effectiveness of traditional antimicrobial drugs, their cost and side effects have led to an increased effort to find other therapeutic approaches. Medicinal plants have always been a mainstay in the management of diseases and are still a major source of healthcare for more than 80% of the population in many developing areas of the world (WHO, 2019). Plants produce a large number of bioactive secondary metabolites such as flavonoids, alkaloids, tannins, saponins, phenolics and terpenoids which have antimicrobial, antioxidant and immunomodulatory properties (Cowan, 1999; Mbuni *et al*., 2020).

The mode of action of these compounds is likely to be multifaceted, including disruption of microbial cell membranes, inhibition of enzyme activity, interference with nucleic acid synthesis and suppression of virulence factors.

Although many studies have reported the antimicrobial activity of plant extracts, more recent studies have been directed towards the synergistic interactions between plant derived compounds. Synergy is when two or more agents have a combined effect that is greater than the sum of its parts (Hemaiswarya *et al*., 2008). Synergistic plant combinations can increase the inhibition of microbes, decrease the amount of the plant needed for the effect, slow the development of microbial resistance, and reduce toxicity in antimicrobial therapy (Cheesman *et al*., 2017). This is especially true in traditional medicine where mixtures of medicinal plants are used, without scientific evidence of the interactions between these plants.

Carica papaya (Caricaceae) and Senna didymobotrya (Fabaceae) are popularly used in Kenyan ethnomedicine for treating infectious and inflammatory diseases. Carica papaya is reported to have antibacterial, antifungal, antioxidant and immunostimulatory properties, which can be attributed to its phytochemical composition which includes papain, flavonoids, alkaloids and phenolic compounds (Duru *et al*., 2019; Singh *et al*., 2020). Likewise, Senna didymobotrya has been used traditionally for treatment of bacterial and fungal infections, gastrointestinal disorders and febrile illnesses and is known to contain bioactive compounds including anthraquinones, saponins, tannins and terpenoids (Jeruto *et al*., 2008; Mworia *et al*., 2021).

Although these plants have been shown to have antimicrobial activity when used alone, there is a lack of scientific evidence of the combined and/or synergistic antimicrobial activity of these plants, especially against clinically relevant pathogens like S. aureus, S. pyogenes and C. albicans. Since traditional use of herbal combinations is common in Kenya and other parts of sub-Saharan Africa, there is a need to scientifically assess the efficacy and therapeutic potential of herbal combinations.

The present study was thus aimed at assessing the synergistic antimicrobial activity of ethanolic extract of Carica papaya and Senna didymobotrya against selected clinically important bacterial and fungal pathogens. This study aims to offer experimental data on their combined effects, which will help in the development of evidence-based plant-derived antimicrobial therapies and rational use of medicinal plants in the era of increasing antimicrobial resistance.

1.1 Research Objective

To determine Synergistic Antimicrobial Activity of Ethanolic Extracts of *Carica Papaya* and *Senna Didymobotrya* Against Selected Human Pathogens in Western Kenya

The methodology used (phytochemical screening, agar well diffusion assays, and statistical comparison of individual and combined extract activities), interpretation of results, and conclusions and recommendations in this manuscript were directly guided by this objective.

II. MATERIALS & METHODS

2.1. Study Area and Plant Collection

Seeds of the plants were gathered from specific locations in the Mount Elgon ecosystem and from sub-counties of Bungoma West, Kanduyi and Webuye West in Bungoma County, western Kenya (Figure. 1). The study area is situated on the western slopes of Mount Elgon and has fertile volcanic soils, moderate to high annual rainfall and a predominantly tropical highland climate. High species diversity of plants and extensive ethnomedicinal use is associated with these environmental conditions.

Geographically, it is situated between 0°25′ N and 1°15′ N and 34°20′ E and 35°05′ E and altitudinal gradients from about 1200m to more than 4300m above sea level towards the Mount Elgon massif. This type of environmental heterogeneity has been shown to affect plant distribution patterns and biosynthesis of secondary metabolites.

The mature and healthy plants were sampled at the proper harvesting season. To minimise intra-population bias and to provide representative spatial coverage, seeds were randomly collected from several individual plants at each sampling site. Each sampling location was georeferenced with a handheld Global Positioning System (GPS) receiver and elevation was measured. The sampling sites are detailed in Table 1 and shown in Fig. 1, and include site codes, geographical coordinates, altitude and habitat characteristics.

The botanical identification and authentication was done by a qualified taxonomist using standard morphological identification keys. Voucher samples were collected and stored in a recognized herbarium for future reference. The collected seeds were cleaned for the removal of extraneous materials and air dried at room temperature (25-28 °C) under shaded well-ventilated conditions until constant weight was reached. The dried materials were ground in a laboratory grinding machine to a fine powder and kept in airtight containers at room temperature for further analysis.

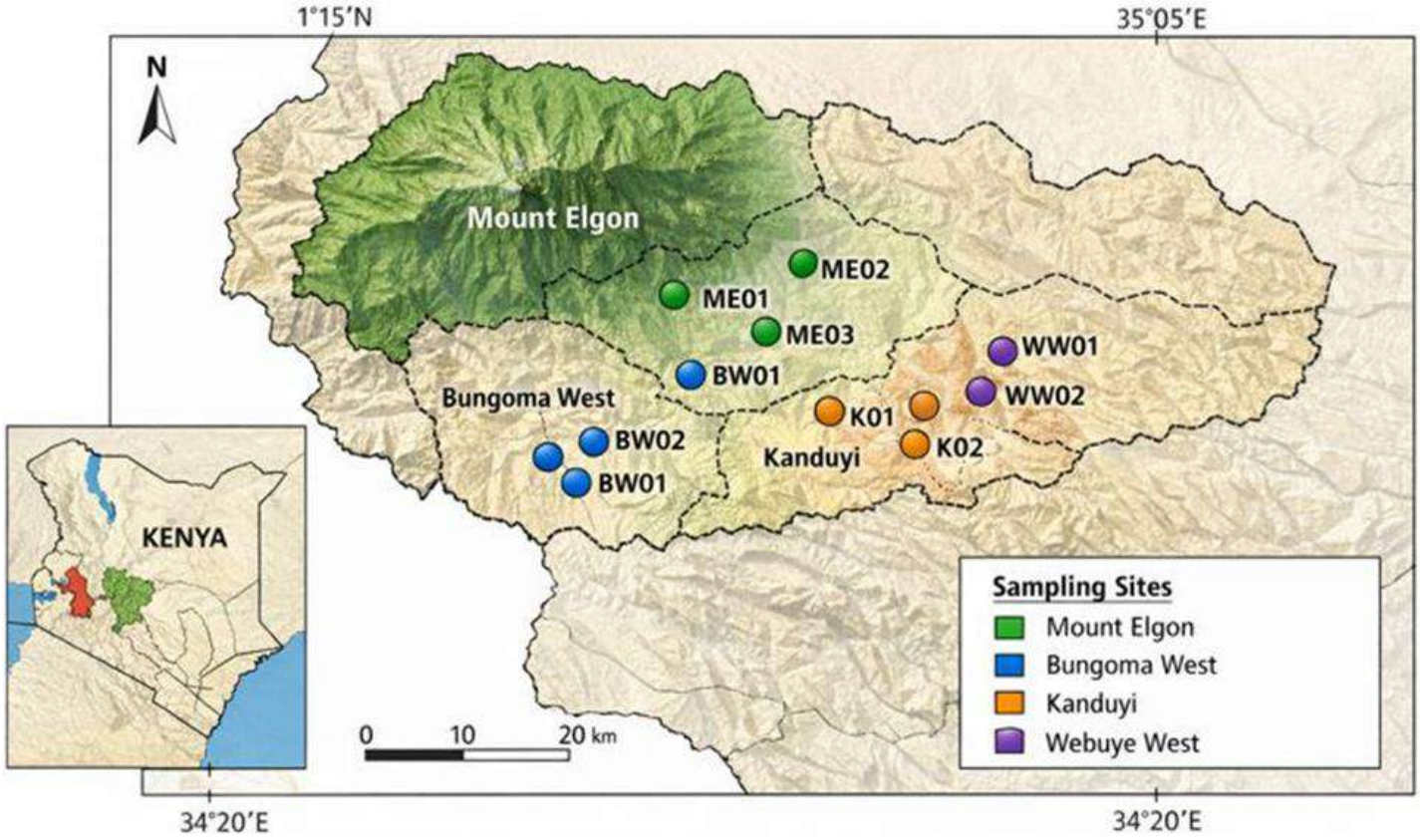


Figure I

Geographical location of the study area in Bungoma County, western Kenya, showing the Mount Elgon ecosystem, and the sub-counties of Bunguma West, Kanduyi and Webuye West. Sampling points are marked where plant seeds were sampled.

Table I

Geographical coordinates and characteristics of plant seed sampling locations in Bungoma County, western Kenya.

Site ID	Sub-county / Ecosystem	Latitude (N)	Longitude (E)	Altitude (m a.s.l.)	Sample Type	Notes
ME01	Mount Elgon ecosystem	0°52′12″	34°39′45″	2,180	Seeds	Highland forest margin
ME02	Mount Elgon ecosystem	0°55′28″	34°42′10″	2,350	Seeds	Upper montane zone
ME03	Mount Elgon ecosystem	0°50′41″	34°41′02″	2,050	Seeds	Mid-slope ecosystem
BW01	Bungoma West	0°34′18″	34°34′56″	1,480	Seeds	Smallholder farmland
BW02	Bungoma West	0°36′02″	34°36′21″	1,520	Seeds	Roadside/agro-ecological zone
K01	Kanduyi	0°33′45″	34°38′40″	1,430	Seeds	Peri-urban cultivation area
K02	Kanduyi	0°35′11″	34°40′05″	1,460	Seeds	Mixed cropping system
WW01	Webuye West	0°37′29″	34°45′12″	1,390	Seeds	Lowland agricultural zone
WW02	Webuye West	0°38′54″	34°46′48″	1,410	Seeds	Riverine-influenced area

2.2. Preparation of Ethanolic Extracts

The dried seeds of each plant species were ground into fine powder using mechanical grinder to increase the surface area and improve the extraction efficiency. The powdered materials were kept in dark and airtight containers at room temperature to avoid moisture uptake and degradation of bioactive components (Harborne, 1998).

To extract, 100 g of the powdered seed material from each plant species was accurately weighed with analytical balance and placed in sterile conical flasks. Each sample was macerated in 500 mL of 95% (v/v) ethanol, making sure that the plant material was fully covered. Ethanol was chosen as the extraction solvent because it extracts a variety of phytochemicals which are polar and moderately non-polar, is relatively non-toxic and is suitable for pharmacological and antimicrobial studies (Eloff, 1998; Cowan, 1999).

Maceration was performed at room temperature (25 ± 2 °C) for 24 h with occasional manual shaking to allow penetration of the solvent into the plant tissues and to increase mass transfer of soluble secondary metabolites into the extraction medium. Maceration at ambient temperature is commonly employed in ethnopharmacological studies because it allows for the least amount of thermal degradation of heat sensitive compounds and closely mimics the traditional methods of herbal preparation (Houghton & Raman, 1998; WHO, 2011).

The mixtures were filtered through Whatman No. 1 filter paper after the extraction period to remove the plant residues which were not soluble. The filtrates obtained were concentrated under reduced pressure in a rotary evaporator to remove the solvent without altering the thermolabile bioactive compounds. When rotary evaporation was not possible, the solvents were removed by evaporation at room temperature under a fume hood. Crude ethanolic extracts were also dried to constant weight to ensure that all the solvent was removed (Harborne, 1998).

The dried extracts were then placed in sterile labeled containers and stored at 4 °C until further use to preserve the stability and biological activity of the extracts. Prior to antimicrobial assays, each crude extract was reconstituted in 1% (v/v) dimethyl sulfoxide (DMSO) to obtain the desired test concentrations. The high solvating capacity of dimethyl sulfoxide and its low antimicrobial activity at low concentrations (Cowan, 1999) made it the ideal choice as a solubilizing agent. To ensure consistency, stability and reproducibility of the antimicrobial activity, all extract solutions were freshly prepared just before testing.

2.3 Test Microorganisms

Staphylococcus aureus, Streptococcus pyogenes and Candida albicans clinical isolates were collected from microbiology laboratories in Maseno University that are accredited. The isolates were manipulated in standard biosafety conditions and identified using conventional morphological, cultural and biochemical methods following standard microbiological procedures (Cheesbrough, 2010; Forbes *et al*., 2016).

The bacterial isolates (Staphylococcus aureus and Streptococcus pyogenes) were kept on nutrient agar slants and Candida albicans on Sabouraud dextrose agar (SDA). Before antimicrobial susceptibility testing, the organisms were subcultured onto fresh media and incubated at 37 °C for 18-24 hours for bacterial isolates and 28-30 °C for 24-48 hours for the fungal isolate to provide actively growing cultures (Cheesbrough, 2010).

Standardized inocula were made by suspending colonies from fresh cultures in sterile normal saline. The turbidity of each suspension was adjusted to correspond to that of the 0.5 McFarland standard, which is approximately 1.5×10^8 CFU/mL for bacterial isolates and 1.0×10^6 CFU/mL for Candida albicans. To ensure accuracy, reproducibility and comparability of results, antimicrobial susceptibility testing was performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2023).

2.4 Phytochemical Screening

The ethanolic extracts of the seeds were subjected to qualitative phytochemical screening for the presence of flavonoids, tannins, saponins, alkaloids, glycosides, phenols and terpenoids by standard biochemical methods. All tests were repeated three times to make sure the results were reproducible and reliable.

The presence of flavonoids was detected by the alkaline reagent test, where a part of the extract was treated with sodium hydroxide solution. The presence of flavonoids (Harborne, 1998) was shown by the formation of an intense yellow coloration which was removed by the addition of dilute hydrochloric acid. Ferric chloride test was used for identification of tannins. The extract was treated with a few drops of 5% ferric chloride solution and the formation of blue-black/greenish colour was considered as an indication of tannins (Sofowora, 1993).

The frothing test was used to detect the presence of saponins. The extract was shaken vigorously with distilled water and the froth formed remained stable for several minutes, which is an indication of the presence of saponins (Evans, 2009).

Alkaloids were tested using diluted hydrochloric acid and then Dragendorff's and Mayer's reagent were added to the extract. An orange-red or creamy precipitate was formed indicating the presence of alkaloids (Harborne, 1998). The Keller–Killiani test was used for the screening of glycosides. The extract was treated with glacial acetic acid containing ferric chloride and concentrated sulfuric acid was then carefully added to it. A brown ring was observed at the interface, which was an indication of the presence of cardiac glycosides (Sofowora, 1993).

The presence of phenols was determined by adding few drops of Ferric Chloride solution to the extract. A deep blue/green colour was indicative of the presence of phenolic compounds (Evans, 2009). The Salkowski test was used for the identification of terpenoids. The extract was then mixed with chloroform and gently overlaid with concentrated sulfuric acid. The presence of terpenoids was confirmed by the formation of a reddish brown colour at the interface (Harborne, 1998).

2.5 Antimicrobial and Synergy Testing

The antimicrobial activity of the ethanolic extracts of the seeds was assessed by the agar well diffusion method, according to standard microbiological procedures (Cheesbrough, 2010; CLSI, 2023). The test was performed with standardised inocula of Staphylococcus aureus, Streptococcus pyogenes and Candida albicans as described in Section 2.3. Bacterial isolates were grown on sterile Mueller Hinton agar (MHA) plates and Candida albicans was grown on Sabouraud dextrose agar (SDA) plates. The microbial suspension was uniformly spread on each agar plate using a swab in three directions to ensure even distribution of the test organism. Inoculated plates were left to stand for about 15 minutes to allow the inoculum to be absorbed.

Wells were aseptically punched in the agar with a sterile cork borer with a diameter of 6 mm. The ethanolic extracts were evaluated alone and in combination at four different concentrations (25%, 50%, 75% and 100% v/v). Each extract solution was carefully dispensed in the appropriate wells in a measured volume (usually 50-100µL). The negative control was 1% DMSO and where applicable, standard antimicrobial agents were used.

Bacterial isolates were incubated at 37 °C for 18-24 hours and Candida albicans at 28-30 °C for 24-48 hours. Antimicrobial activity was determined by measuring the zones of inhibition around each well after incubation with a transparent ruler or digital caliper. The zone diameters were measured in millimeters (mm) and the tests were repeated three times. The average ZI values were calculated and reported.

Synergistic activity was determined by comparing the zones of inhibition of the combined extracts with the zones of inhibition of the individual extracts at the same concentrations. Synergy was indicated if the inhibitory effect of the combined extracts was significantly more than the effect of the individual extracts, and antagonism or indifference was indicated if there was no enhancement or a reduction in activity (Odds et al., 2003; Chanda & Rakholiya, 2011).

2.6. Data Analysis

Each experiment was repeated three times and the data presented as mean ± standard deviation (SD). The data collected from the antimicrobial assays such as the zones of inhibition at various extract concentrations (25%, 50%, 75% and 100%) were first organized and tabulated before statistical evaluation.

Descriptive statistics

The mean zone of inhibition ($\bar{x}$) of each treatment group was computed by:

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x_i \tag{1}$$

where x_i represents individual measurements and nis the number of replicates.

The standard deviation (SD) of each set of data was calculated as:

$$SD = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}} \tag{2}$$

Central tendency and dispersion of the antimicrobial activity data were summarized by descriptive statistics.

2.6.1 Inferential Statistical Analysis

To compare the antimicrobial effect of the extracts at different concentrations (25%, 50%, 75% and 100%) one-way analysis of variance (ANOVA) was used to determine if there were any statistically significant differences between the group means (Field, 2013; Kirkwood & Sterne, 2003). The ANOVA F-statistic was computed to be:

$$F = \frac{MS_{\text{between}}}{MS_{\text{within}}} \tag{3}$$

where MS_{between} is the mean square between the treatment groups and MS_{within} is the mean square within the groups.

If the ANOVA showed that there were significant differences between the means ($p<0.05$), then the appropriate post hoc multiple comparison tests were conducted to determine which groups differed from each other, while controlling for Type I error (Field, 2013).

2.6.2 Comparison of the Individual and Combined Extracts

Independent sample t-tests were performed to compare the antimicrobial activities of individual extracts to the combined extracts to determine if there were any synergistic effects (Kirkwood & Sterne, 2003). The t-statistic was calculated as:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}} \quad (4)$$

Here $\bar{x}_1$ and $\bar{x}_2$ are the group means, s_1^2 and s_2^2 are the variances and n_1 and n_2 are the sample sizes.

2.6.3 Significance Threshold

Standard statistical software was used for all statistical analyses. Statistically significant differences were set at $p<0.05$ (95% confidence level). The statistical procedures were done according to the accepted biostatistical guidelines for biomedical and microbiological research (Field, 2013).

III. RESULTS & DISCUSSION

3.1 Results

3.1.1 Phytochemical Composition

Qualitative phytochemical screening of the ethanolic extracts of the seeds showed that there were several major classes of secondary metabolites in both *Carica papaya* and *Senna didymobotrya* in all the sampling locations. Phytochemical constituents found were flavonoids, tannins, saponins, alkaloids, glycosides, phenolic compounds and terpenoids (Table 2).

The presence of these phytochemical classes was constant for each plant species, with no difference in the phytochemical profile of the plants collected from the various sites, suggesting a stable qualitative phytochemical profile for each plant species. No qualitative difference was found between the two species of plants or between the samples from the different ecological zones in the study area. This homogeneity indicates that the biosynthesis of secondary metabolites is conserved in each species under the environmental conditions.

Of particular importance are the presence of flavonoids and phenolic compounds, which have been well documented to possess antioxidant and antimicrobial properties, many of which have been linked to membrane disruption, enzyme inhibition and metal ion chelation. Tannins and saponins are also known to play a role in antimicrobial activity, by precipitating proteins and increasing cell membrane permeability, respectively. Alkaloids and terpenoids are known to possess a wide spectrum antimicrobial activity and glycosides might exert indirect antimicrobial activity by liberating bioactive aglycones.

The qualitative phytochemical profile obtained in this study generally gives a biochemical explanation for the antimicrobial activities of the ethanolic seed extracts tested in the subsequent assays. The results were, however, qualitative, and only show the presence or absence of these compounds, not their relative concentrations.

Table II

Phytochemical composition of the ethanolic extracts of Carica papaya and Senna didymobotrya seeds was determined qualitatively.

Phytochemical class	***C. papaya***	***S. didymobotrya***
Flavonoids	+	+
Tannins	+	+
Saponins	+	+
Alkaloids	+	+
Glycosides	+	+
Phenols	+	+
Terpenoids	+	+

Key: (+) Present

3.1.2 Individual Antimicrobial Activity

The ethanolic extracts of Carica papaya and Senna seeds showed concentration dependent antimicrobial activity against all the microorganisms tested. The lowest concentration (25%) showed no inhibition while measurable zone of inhibition was obtained at 50%, 75% and 100% concentration for all the test organisms (Table III).

The maximum mean ZOI was always recorded against Streptococcus pyogenes followed by Staphylococcus aureus and comparatively low activity was recorded against Candida albicans. The antimicrobial activity of the extracts was found to be positively correlated with the concentration of the extracts for both the plant species (Figure III).

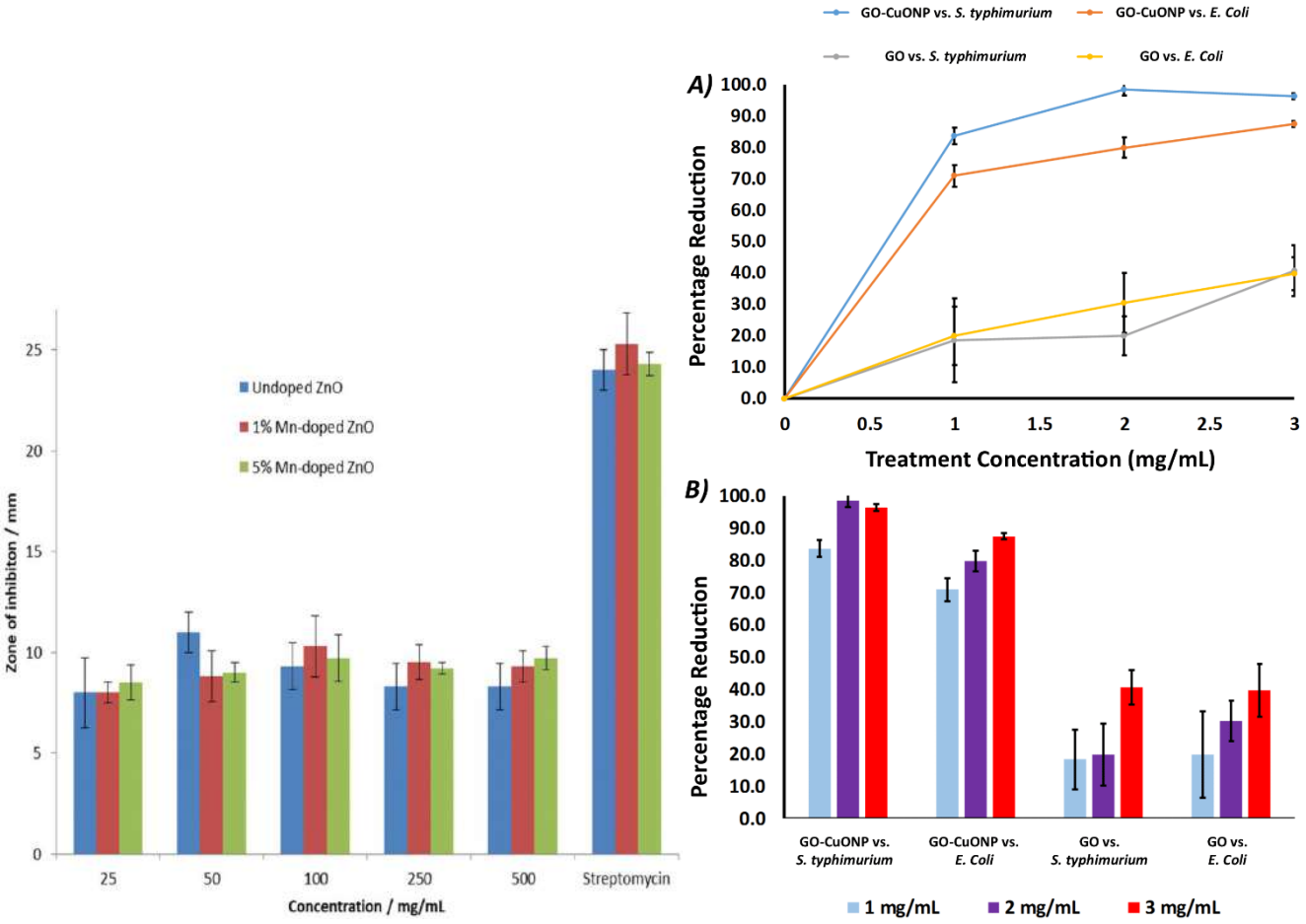
Both the extracts had good antibacterial activity with *S. didymobotrya* extracts showing slightly larger inhibition zones at the highest concentration (100%). Both extracts showed moderate antifungal activity against *C. albicans* which was significantly enhanced with concentration.

The antimicrobial activity of the extracts for all test organisms and both plants varied significantly with extract concentration ($p < 0.05$) as determined by one-way analysis of variance (ANOVA). The high F-values obtained show that the differences in inhibition zones were mostly due to the differences in extract concentration and not due to random variation (Table 3).

Table III

Zones of inhibition (mm) of the individual ethanolic seed extracts against test microorganisms (mean ± SD).

Organism	Extract	25%	50%	75%	100%	ANOVA (F, p)
S. pyogenes	*C. papaya*	0.0 ± 0.0	12.4 ± 0.6	16.8 ± 0.7	20.3 ± 0.5	F = 48.7, p < 0.001
	S. didymobotrya	0.0 ± 0.0	13.1 ± 0.5	17.5 ± 0.6	21.0 ± 0.4	F = 52.3, p < 0.001
S. aureus	*C. papaya*	0.0 ± 0.0	10.6 ± 0.4	14.2 ± 0.6	18.0 ± 0.5	F = 41.9, p < 0.001
	S. didymobotrya	0.0 ± 0.0	11.3 ± 0.5	15.1 ± 0.5	18.7 ± 0.6	F = 44.6, p < 0.001
C. albicans	Both extracts	0.0 ± 0.0	7.2 ± 0.3	9.4 ± 0.4	11.6 ± 0.5	F = 26.8, p < 0.001



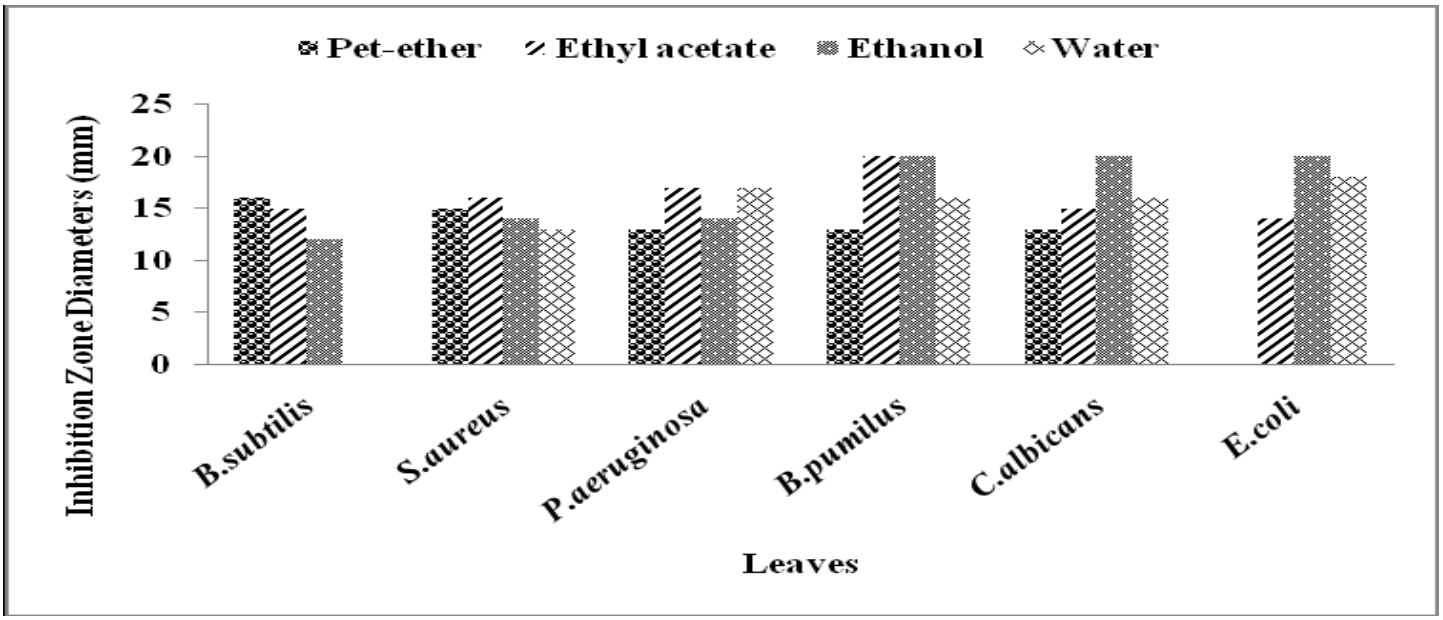


Figure II

Antimicrobial activity of Carica papaya and Senna didymobotrya ethanolic seed extracts at different concentrations against test microorganisms. Each bar represents the mean ± standard deviation (SD) of the zone of inhibition (mm) at different extract concentrations.

The figure A shows the antibacterial activity of graphene oxide–copper oxide nanoparticles (GO–CuONP) and graphene oxide (GO) against Salmonella typhimurium and Escherichia coli as a percentage reduction, which is concentration dependent. The GO–CuONP showed significantly better antibacterial activity against both bacterial strains at all tested concentrations (1–3 mg/mL) compared to GO alone.

The percentage reduction was found to be significantly increased at 1 mg/mL for GO–CuONP, especially against S. typhimurium and then almost maximum inhibition was observed at 2 mg/mL and 3 mg/mL. However, GO alone showed significantly less antibacterial activity at all concentrations, suggesting that GO does not have much intrinsic antibacterial activity. In general, S. typhimurium showed a slight susceptibility towards GO–CuONP treatment as compared with E. coli.

Comparative bar-chart summary of antibacterial activity at discrete concentrations (1, 2 and 3 mg/mL) is shown in Figure B. As in Figure A, GO–CuONP showed significantly higher antibacterial activity than GO at all concentrations for both bacterial species. The highest activity was seen at 3mg/mL concentration where GO–CuONP showed almost complete reduction of the bacteria while GO showed moderate inhibition. These results indicate the synergistic effect of the incorporation of CuO nanoparticles in the graphene oxide matrix for enhancing antibacterial activity.

Figure C: The second figure shows the antimicrobial activity of leaf extracts obtained from various solvents (petroleum ether, ethyl acetate, ethanol and water) on various test microorganisms in terms of inhibition zone diameters (mm).

In all the organisms tested, ethanol extracts gave the highest zone of inhibition which means that it had the best extraction of bioactive antimicrobial compounds. The extracts of ethyl acetate also exhibited moderate antimicrobial activity while the extracts of petroleum ether and aqueous generally exhibited lower inhibitory effects.

Bacillus pumilus, Candida albicans and *E. coli* were the most sensitive test organisms with inhibition zones of about 20 mm for ethanolic extracts. The relatively weak activity of the aqueous extracts indicates that a significant number of the antimicrobial active compounds are more soluble in organic solvents, especially ethanol.

It is concluded that the polarity of the solvent is an important factor in the antimicrobial activity of the plant extracts and ethanol was found to be the best extraction solvent for the extraction of antimicrobial phytochemicals from leaf material.

3.1.3. Synergistic Antimicrobial Effects

The ethanolic seed extracts of *Carica papaya* and *Senna didymobotrya* showed a combined effect on the antibacterial activity against *Streptococcus pyogenes* and *Staphylococcus aureus* as compared to the individual extracts. The combined extracts at 50%, 75% and 100% showed significantly bigger ZOI against both the bacteria species, suggesting that there was a synergy between the two plant extracts (Table 4).

Independent sample t-tests showed that the increase in antibacterial activity of the combined extracts was statistically significant for S. pyogenes and S. aureus ($p < 0.05$). Furthermore, the antimicrobial activity of the combined extracts was found to be concentration dependent as revealed by one-way analysis of variance (ANOVA) which showed a significant effect of extract concentration. The antibacterial activity of the combined extracts is shown in Figure III, which shows that the combined extracts are more active than the individual extracts.

However, no synergistic effect was seen against *Candida albicans*. The inhibition zones of the combined extracts and the individual extracts of all concentrations against this organism did not significantly differ ($p > 0.05$), indicating that the interaction of the phytochemical constituents was not enough to enhance the antifungal activity.

In general, these findings indicate that the combined extracts exhibited synergistic effect which was organism-specific and was more apparent against the Gram-positive bacterial pathogens as compared to the fungal isolate tested.

Table IV:

Comparison of antimicrobial activity of individual and combined ethanolic seed extracts (mean zone of inhibition ± SD, mm)

Organism	Treatment	50%	75%	100%	Statistical outcome
S. pyogenes	Individual	12.8 ± 0.6	17.2 ± 0.7	20.6 ± 0.5	—
	Combined	15.6 ± 0.5	20.1 ± 0.6	24.3 ± 0.4	*t*-test, $p < 0.01$
S. aureus	Individual	11.0 ± 0.5	14.6 ± 0.6	18.4 ± 0.6	—
	Combined	13.8 ± 0.4	18.2 ± 0.5	22.1 ± 0.5	*t*-test, $p < 0.01$
C. albicans	Individual	7.2 ± 0.3	9.4 ± 0.4	11.6 ± 0.5	—
	Combined	7.5 ± 0.4	9.6 ± 0.5	11.8 ± 0.4	$p > 0.05$

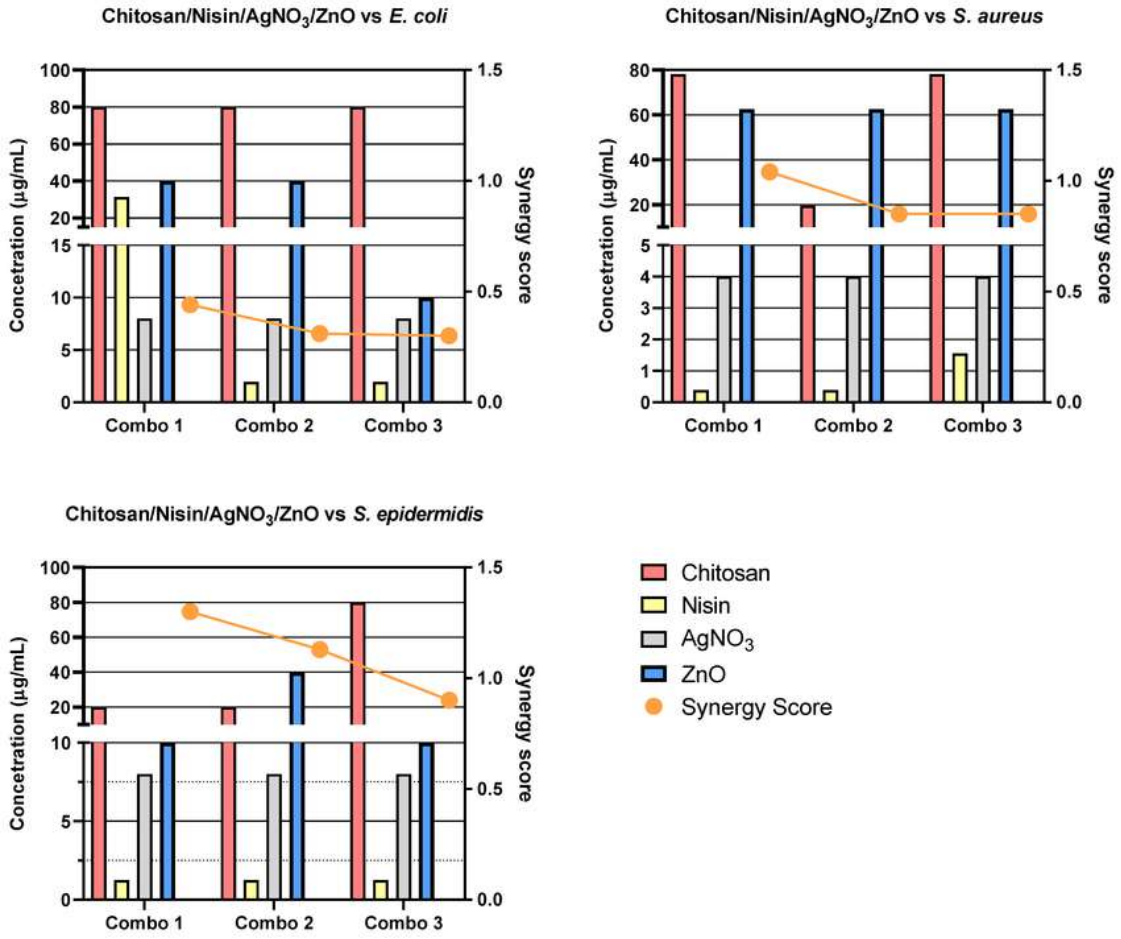


Figure III

Antimicrobial activity of the individual and combined ethanolic seed extracts of Carica papaya and Senna didymobotrya against the test microorganisms. The mean zone of inhibition (mm) ± standard deviation (SD) at different extract concentrations are represented by bars, showing that the combined extracts were more effective against the bacterial isolates.

3.2 Discussion

The present study showed that the combined ethanolic extract of *Carica papaya* and *Senna didymobotrya* had a better antibacterial activity against *Streptococcus pyogenes* and *Staphylococcus aureus* than single extracts, suggesting that the two extracts were synergistic. The observed synergism may be due to complementary or additive effect of the phytochemical constituents present in both the plants which were qualitatively detected in all the extracts such as flavonoids, alkaloids, tannins and terpenoids.

Flavonoids and phenolic compounds have been widely reported to have antibacterial activity in a variety of ways, such as disrupting bacterial cell walls and membranes, inhibiting nucleic acid synthesis and interfering with energy metabolism (Cowan, 1999; Cushnie & Lamb, 2011). Alkaloids, however, have been found to inhibit important enzymes of bacteria and disrupt cell division, and tannins have been found to precipitate membrane proteins and enzymes, which inhibit growth or kill bacteria (Daglia, 2012). These phytochemicals, when found in combination, may target different cells at the same time and thus increase the efficacy of the antibacterial activity and minimize the chance of bacterial survival.

The very strong synergistic activity found against the Gram-positive bacteria *S. pyogenes* and *S. aureus* could also be attributed to the structural characteristics of the cell walls of these organisms. Gram-positive bacteria do not have an outer membrane like Gram-negative bacteria and have a thick peptidoglycan layer which may make them more susceptible to phytochemicals that target cell wall integrity and membrane permeability (Silhavy *et al*., 2010). This could be one of the reasons why the combined extracts in this study had a higher susceptibility to these organisms.

The lack of synergistic antifungal effects against Candida albicans, however, indicates organism specificity of the combined phytochemicals. Fungal cells have a more complex cell wall made up of chitin, β-glucans, mannoproteins and a cell membrane containing ergosterol instead of cholesterol (Odds *et al*., 2003). These structural and biochemical differences may provide intrinsic resistance to some plant derived compounds and/or restrict the activity of combinations of phytochemicals that are active against bacteria. Furthermore, C. albicans is also known to have adaptive resistance mechanisms such as efflux pumps and biofilm formation that can decrease susceptibility to antimicrobial agents (Cowen *et al*., 2015).

The results obtained in this study corroborate with the existing evidence of the use of polyherbal formulations to increase the antibacterial activity due to synergistic interactions but also recognise that this does not apply to all groups of microbes (Eloff *et al*., 2017). A number of studies have found that mixtures of plant extracts can be more effective in achieving antibacterial activity, especially against Gram-positive pathogens, and that antifungal activity is mostly moderate and additive, with no synergism (van Vuuren & Viljoen, 2011).

Notably, the observed synergy between the two herbs against *S. pyogenes* and *S. aureus* highlights the potential of using herbal combinations for treating bacterial infections, particularly in light of the growing concern over antimicrobial resistance. Plant-based combinations could be an alternative to traditional antibiotics, which act on one pathway of the bacteria, and so lessen the development of resistance. However, more studies are needed to validate and optimize such combinations for clinical use, including quantitative phytochemical analysis, determination of minimum inhibitory concentrations and elucidation of specific mechanisms of action.

IV. CONCLUSION & RECOMMENDATIONS

4.1 Conclusion

The results obtained in this study revealed that ethanolic extract of *Carica papaya* and *Senna didymobotrya* seeds showed significant concentration dependent antimicrobial activity and when applied in combination, showed remarkable synergistic antimicrobial activity against Streptococcus pyogenes and Staphylococcus aureus. By contrast, no synergistic antifungal activity was seen against *Candida albicans*, indicating that there are fundamental differences between the cellular targets of bacteria and fungi. This observed synergy between the antibacterial activity of the two plants may be due to complementary action of the phytochemical constituents like flavonoids, alkaloids, tannins and phenolics found in both the plants. These interactions can potentially increase antibacterial activity by acting on multiple targets such as interfering with the integrity of the cell wall and blocking the metabolic pathways. The absence of synergism against *C. albicans* indicates that the structure of the microbes, resistance mechanisms and vulnerabilities of pathogens should be taken into account when assessing combined plant extracts. These findings have implications not only for the pharmacological aspects, but also for disaster risk management and public health resilience in low-resource and disaster-prone regions, where access to conventional antibiotics might be restricted as a result of the disruption of supply chains, disease outbreaks, or humanitarian emergencies. Community level preparedness may be achieved by using antimicrobial combinations derived from plants as

locally available, cost-effective complementary interventions during health crises, such as epidemics and post-disaster situations where bacterial infections are common.

4.2 Recommendations

There is a need to incorporate the scientifically validated combination of C. *papaya* and S. *didymobotrya* ethanolic extracts into national antimicrobial resistance and health-resilience strategies as complementary interventions to minimize the use of antibiotics and enhance preparedness in resource-limited settings. Locally available antimicrobial plant materials that have been proven to be effective should be included in disaster preparedness and emergency health response plans to reduce the risk of infection during disasters and supply-chain disruptions. Policy and academic efforts should be encouraged to document indigenous knowledge and manage the use of medicinal plants in an ethical and sustainable manner for long-term public health security and ecological resilience.

Declaration of Interest

The authors declare that they do not have any known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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