Accessing the potential of the African spider plant (*Cleome gynandra*) to control *Salmonella typhi*

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Abstract

The African spider plant *(Cleome gynandra)* is an erect tender annual herb that is highly branched. This popular and nutritious leafy vegetable has been commercialized as a major African indigenous vegetable in Kenya. The African spider plant has also been an antidote for typhoid and poor sanitation-related illnesses. *Salmonella typhi* is common with an estimated annual global incidence of 26.9 million cases, and causes 200,000 deaths annually due to mismanagement of water resources and poor sanitation. To validate the medicinal myth, this study aimed to evaluate the efficacy of crude extracts from the African Spider plant, *Cleome gynandra* against *Salmonella typhi* the leaf extracts of this vegetable were tested for activity against cultures of *Salmonella typhi* haplotype H58 in vitro. Fresh leaves were homogenized in sterile distilled water (SDW) at 1:2 wt/vol ratios. Extraction was done overnight at 4°C and then centrifuged at 1500rpm for 5 minutes. The supernatant was sterilized through 0.2µm Millipore filters and diluted up to x10 $^{-3}$. About 40µlof each diluted extract was loaded onto sterile discs and allowed to air dry under sterile conditions. Similar volumes of Ampicillin at 10µl and SDW were used as positive and negative controls respectively. About 100µl of x10 $^{-7}$ diluted suspensions of *Salmonella typhi* were inoculated and spread dry on Muller Hilton agar plates. The preloaded discs were then placed radially at a 6cm circumference on the inoculated 9cm Petri plates. The plates were then incubated at 37°C for 24 hours and growth inhibition zones measured (mm) values were compared statistically using one-way ANOVA to the controls and inference made. All the test crude extracts suppressed the growth of Salmonella typhi significantly compared to SDW controls (p=0.00000136). Straight extracts at 20mg bioactive compound had significantly high (p=0.00000136) bioactivity compared to x10 $^{-1}$ and x10 $^{-2}$ dilutions but significantly lower than the Ampicillin control. Interestingly, extracts diluted to x10 $^{-2}$ with 5mg bioactive compounds suppressed salmonella typhi significantly (p< 0.001). *Cleome gynandra* has therefore demonstrated antibacterial properties against *Salmonella typhi*, thus gradually utilizing these plant leaves at high concentrations is recommended to suppress the infections caused by *Salmonella typhi* pathogenic bacteria.

Key Words: Medicinal indigenous vegetables, African spider plant, antimicrobial activity, typhoid.

Introduction

The African spider plant (*Cleome gynandra*) is an erect tender annual herb growing to about 1.4 meters in height. It belongs to the *Capparaceae* family (formerly *Capparidaceae*), subfamily *Cleomaceae* (Lokesha, 2018). There are about 700-800 species, divided into 45 general fields (Munene et al., 2018). Traditionally the plant has been used as a vegetable in the western region of Kenya, it has been used to treat scurvy and stomachaches, and in pregnant mothers, it can be used to reduce dizziness (Phytochemical, 2017). It is also used in the treatment of bronchitis, boils, nasal congestion, and diarrhea (Muhialdin et al., 2018). The bacteria that cause typhoid (*Salmonella typhi*) is spread through contaminated food or water through the fecal-oral route. Symptoms of the disease include; high fever, headache, abdominal pain, stomachache, constipation, or diarrhea. Typhoid poses a significant public health burden in Kenya, with 97,762 cases and 1,075 deaths reported in 2016 with 62% and 66% of these cases reported found among children aged 15 years or less (Simiyu & Jamka, 2018).

The use of plant compounds for pharmaceutical purposes has gradually increased in the world (Salmerón-Manzano et al., 2020). According to recent research, medicinal plants would be the best source to obtain a variety of drugs (Bhat, n.d.). The lack of toxicity of many plants has been scientifically proven in recent years (Mensah et al., 2019). The global scenario is now changing towards the use of nontoxic plant products having traditional medicinal use (Ss & Sk, 2011). The development of modern drugs from plants should be emphasized for the control of various diseases (Ekor, 2014). Typhoid infections are a noxious disease because it is often misdiagnosed and treated as malaria which makes it persistent in the community. The treatment is painful, expensive, and long and the possibility for re-infection is very high during or after treatment (Dyson et al., 2019). Given the traditional use of medicinal plants as a source of medicine in many diseases, folklore, and claims made by the people in different Kenyan communities that *Cloeme gynandra* leaves have traditionally been used to treat stomaches, scurvy, constipation, and diarrhea (Muhialdin et al., 2018). The present work has been undertaken to evaluate the antibacterial activities of different extracts of the *Cleome gynandra*.

Salmonella typhi has been a widespread problem in Kenya with Kibera (> 2,000 cases per person-years of observation (Ng'eno et al., 2023) and Western Kenya among the leading regions with high typhoid incidences affecting a larger population due to contaminated water and fish sources (Karkey et al., 2017). In Kenya, there are water problems in rural and urban settings (Mutisya & Yarime, 2011; Thompson & International Institute for Environment and Development, 2001). Most residents rely on open and underground water sources which can easily be contaminated (Ananga et al., 2017; *Tackling Water Pollution In Kenya | African Development Choices*, n.d.). In most urban areas the integrity of the piping system is breached and sometimes contaminated with sewage (Fever & Overview, 2011). Over the rainy season, cases of typhoid are very high in both rural and urban areas due to water contamination (Muthumbi et al., 2015). In both rural and urban areas 50% of the toilet systems are pit latrines (Nakagiri et al., 2016). The pits are deep and, in many cases, they contaminate groundwater (Orner et al., 2019).

Typhoid infections are a noxious disease because it is often misdiagnosed and treated as malaria, making them persistent in the community (*It's Not Typhoid – Tackling Misdiagnosis of Typhoid Fever in Nigeria - Nigeria Health Watch*, n.d.; Meatherall et al., 2014). The treatment is painful, expensive, and long, and the possibility for re-infection is very high during or after treatment (Dyson et al., 2019). For easy management of typhoid introduction of special medication in the form of meals especially vegetables could be an alternative control strategy. For a long time, there has been a myth that the African spider plant possesses medicinal properties and can help prevent typhoid in individuals who frequently consume it (Khan & Kubmarawa, 2015).

African spider plant is used as vegetables either fresh or pickles (*The Spider Plant: A Hardy and Nutritious African Native • We Blog The World*, n.d.). Many societies believe that it has medicinal properties that could ally many ailments when taken as a vegetable (Munene et al., 2018). The leaves when crushed and boiled could help prevent scurvy, leaves boiled and marinated in sour milk make a medicinal meal that could help improve eyesight, provide energy, and cure marasmus (Chataika et al., 2021). Boiled leaves of the plant can be used to cure diarrhea and stomachaches related to typhoid (Kameshwaran & Rajamanickam, 2013). Assessment of the phytochemical components has shown that it has saponins and other biologically active non-toxic compounds which are medically important fields (Ganesh et al., 2018). Though the herb is used as a vegetable, its antibacterial properties specifically against *Salmonella typhi* have not yet been validated (Mashamaite et al., 2022).

In developing countries, conventional antibacterial drugs are not affordable compared with indigenous herbal medicines which are readily available (Wachtel-Galor & Benzie, 2011). Approximately 60-80% of the world's population relies on traditional medicines as remedies for common illnesses (Ekor, 2014). The herbs can be taken continuously as vegetables as opposed to conventional drugs and at the same time provide medicinal properties. A daily intake of more than 400g of vegetables and fruits per person can protect the body against chronic diseases (Gido et al., 2016). The leaves of the African Spider plant do not have any side effects when consumed (Rotich et al., 2023). The plant is also adaptable to most environmental conditions in Kenya and is easy to grow (Chweya et al., 1997; Schippers & Natural Resources Institute (Great Britain), 2000). In addition, there are cases of antibiotic resistance by pathogenic microorganisms to most conventional drugs, and is therefore important to have alternative solutions (Chinemerem Nwobodo et al., 2022; Mancuso et al., 2021). The analysis demonstrates the potential antibacterial properties of *Cleome gynandra*. Future research should explore optimal dosages and long-term effects.

Materials and Methods

Study site

Kilifi, Pwani University farm was chosen as a study based on the prevalence of the African Spider plant growth. The study was conducted at the Department of Biological Sciences at Pwani University, Kilifi, Kenya. The study location offered access to state-of-the-art microbiological labs and a controlled setting essential for thorough testing and analysis.

Sample collection and preparation

The study was carried out at the Pwani University Biology laboratory. One kilogram of the African Spider plant leaves was collected from the Pwani University farm using a sterile pair of scissors and gloves and put in sterile zipped airtight collection bags, it was brought to the lab in a sterile cooler box within one hour of collection. The vegetables were picked in the morning hours, between 8 am and 10 am, to ensure freshness and reduce degradation that may result due to high temperatures. Sterile gloves were worn throughout the process and labels were placed in the collection bags indicating the type of the vegetable and the location of the leaves in the farm. Inside the lab, the working area was sterilized and the leaves were thoroughly washed in a running tap and sterile distilled water simultaneously. 20g of the leaf extract was homogenized in sterile distilled water at a 1:2 w/v ratio.

Extraction of the crude extracts

About 20 grams of the leaves were homogenized in 40 ml of sterile distilled water. Extraction was done overnight at 4^0C, and after 12 hours, centrifugation at 1500rpm for 5 minutes was done (Arullappan et al., 2009; Lukubye et al., 2022; Ricci et al., 2021). The supernatant was then sterilized through 0.2µm Millipore filters under sterile conditions. For bioassay, 100ul of the extract was serially diluted in SDW up to 10^{-2}.

Preparation and loading of sterile paper discs

Paper discs were prepared from Whatman filter paper using a paper punch and then autoclaved at 121^0C for 15 minutes. Under sterile conditions, 40ul of the crude extract was loaded on sterile paper discs and allowed to dry (Shashini Janesha et al., 2020). 10µl of ampicillin solution was loaded on the discs to serve as positive control and 10µl of sterile distilled water was loaded on the discs to serve as the negative control (Arullappan et al., 2009; Ricci et al., 2021). The discs were allowed to dry before loading them on the agar medium.

Media preparation

Following meticulously the guidelines on Standard Operating Procedures for Microbiology, media preparation, and sterilization were done by weighing Muller Hilton agar, dissolving it in distilled water heating it to dissolve then autoclaving it at 121^0C for 15 minutes. Aseptically, the media was poured at a temperature of about 55^0C onto the Petri plates and left to cool. The Petri plates were sterilized by putting them in an oven at 160^0C for 2 hours.

Preparation of bacteria isolates

Bacteria isolates of *Salmonella typhi* were reconstituted at the Pwani University laboratory under the Standard Operating Procedures for Microbiology. A scoop of *Salmonella typhi* colony-grown Eosin Methyl Blue media was suspended in 1ml of Sterile Distilled Water (SDW) and serially diluted up to $10^{-7.}$

Bioassay

About 100ul of suspension drawn from 10^{-7}, $10^{-6,}$ and 10^{-5} of the bacterial dilutions was spread in Muller Hilton agar plates and was dual plated with test extracts. The plates were then sealed with Parafilm under sterile conditions and incubated at 37^0C for 24 hours.

Screening for the antimicrobial resistance of the plant extracts

The bacteria cultures were grown under Muller Hilton agar medium at 37^0C. Subsequently, filter paper discs (6 mm in diameter) saturated either with extract or ampicillin (10µl) were placed on the surface of each inoculated plate. The paper discs containing the leaf extract were plated on the inoculated Petri plates in the order of their concentration, 10^0, 10^1, 10^2, positive control (ampicillin), and negative control (distilled water)(Balouiri et al., 2016) .

After 24 hours, the minimum inhibition zones were measured in millimeters using a 15 cm ruler and recorded. Overall, cultured bacteria with halos equal to or greater than 7mm were considered susceptible to the tested extract. The ampicillin control used was proved to show no inhibitions in previous studies. Extracts that showed antimicrobial activity were later tested to determine the Minimal Inhibitory Concentration (MIC) for the bacterial samples. Analysis of variance was done using Microsoft Excel ANOVA to determine the variance between means of the zones of inhibitions of the three levels of crude extract concentrations on the growth of *Salmonella typhi* (Balouiri et al., 2016).

Results

Evaluation of the antimicrobial potential of the plant extracts

The data for plant extract that showed the antimicrobial potential of the African Spider Plant extracts are provided in Table 1, Table 2, and Figure 1 below;

Table 1 summarizes the antimicrobial efficacy of different concentrations of *Cleome gynandra* leaf extracts on the growth of a test *Salmonella typhi*. The zones of inhibition are measured in millimeters and represent the area around the sample where bacterial growth was inhibited. The positive control is a known antimicrobial agent. The 10^0 concentrations of *Cleome gynandra* showed the highest antimicrobial activity among the tested concentrations, with an average zone of inhibition of 11.0 mm. The 10^{-1} concentration showed moderate activity with an average inhibition zone of 8.33 mm. The 10^{-2} concentration showed the least activity, with an average inhibition zone of 6.67 mm. The positive control exhibited a significantly larger zone of inhibition (19.33 mm on average), indicating its higher antimicrobial effectiveness compared to the plant extracts. The standard deviations and standard errors are relatively low across all concentrations, indicating consistent results within each concentration group. The results indicate that the antimicrobial activity of *Cleome gynandra* is concentration-dependent, with higher concentrations showing greater inhibitory effects on the test microorganism. However, the plant extract's effectiveness is still lower than the positive control, suggesting that while *Cleome gynandra* has antimicrobial properties, it may not be as potent as standard antimicrobial agents.

Figure 1, the bar graph represents the mean zones of inhibition (in millimeters) for different concentrations of *Cleome gynandra* leaf extract and a positive control on the growth of *Salmonella typhi* bacteria. The graph includes error bars indicating the standard error for each treatment. At 10^0 Concentration the mean zone of inhibition is approximately 11 mm and the error bar is relatively small, indicating a low variation around the mean. At 10^{-1} concentration, the mean zone of inhibition is approximately 8.33 mm and the error bar is small, indicating consistent results within this concentration. At 10^{-2} concentration, the mean zone of inhibition is approximately 6.67 mm and the error bar is small, indicating low variability around the mean. The positive control's mean zone of inhibition is approximately 19.33 mm and the error bar is larger than those for the extract concentrations, indicating some variability but still demonstrating a significantly higher mean inhibition zone compared to the extract concentrations. The antimicrobial activity of *Cleome gynandra* extract is clearly concentration-dependent. Higher concentrations (10^0) exhibit larger zones of inhibition compared to lower concentrations (10^{-1} and 10^{-2}). The efficacy decreases as the concentration decreases, with the 10^0 concentration showing the highest mean zone of inhibition.

The positive control shows a significantly larger mean zone of inhibition (approximately 19.33 mm) compared to all concentrations of the plant extract. This indicates that while *Cleome gynandra* extracts have antimicrobial properties, they are less effective than the positive control, which is a standard antimicrobial agent. The small error bars for the extract concentrations indicate that the measurements are consistent and reliable within each concentration group. The larger error bar for the positive control suggests greater variability, but its mean zone of inhibition is still much higher than those of the plant extracts.

The graph demonstrates that *Cleome gynandra* has antimicrobial activity against the test microorganism, with effectiveness increasing with concentration. However, the plant extract is less potent than the positive control, highlighting the need for further research to enhance its efficacy or identify additional bioactive compounds. The results also underscore the importance of concentration in determining the antimicrobial effectiveness of plant extracts.

Table 2: The table provides the results of an ANOVA test conducted to compare the means of the zones of inhibition for three different concentrations (10^0, 10^{-2}, and 10^{-3}) of crude leaf extract on the growth of *Salmonella typhi.* A p-value of 1.36E-06 (0.00000136) was obtained.
The ANOVA results show a statistically significant difference in the zones of inhibition for the different concentrations of crude leaf extract on the growth of *Salmonella typhi*. The very low p-value (1.36E-06) strongly indicates that the observed differences in antimicrobial activity between the extract concentrations are not due to random chance. Therefore, the concentration of the leaf extract significantly affects its antimicrobial efficacy.

Table 1: Table showing the average of zones of inhibition of each concentration of the *Cleome gynandra*.

Treatment	10^0	10^{-1}	10^{-2}	Positive control
	10.0	8.0	7.0	21.0
	12.0	9.0	6.0	19.0
	11.0	8.0	7.0	18.0
Mean	11.0	8.33	6.67	19.33
Standard deviation	0.82	0.47	0.47	1.25
SE	0.47	0.27	0.27	0.72

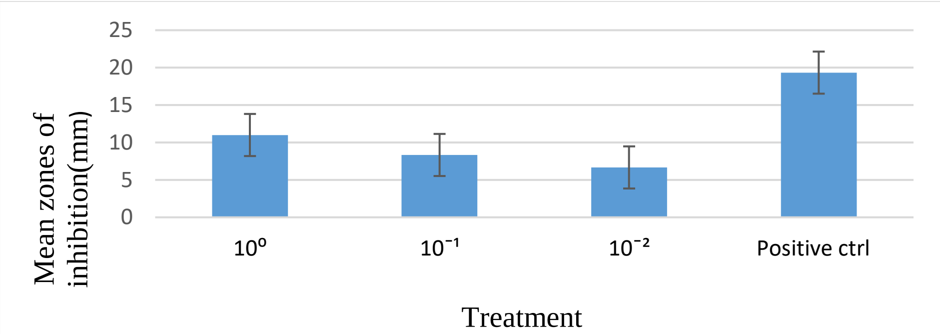

Figure 1: Effects of the different concentrations of leaves crude extract of *Cleome gynandra* on the growth of *Salmonella typhi*

Table 2: Microsoft Excel Analysis of Variance (ANOVA) table between the means of the zones of inhibition of the 10^0, 10^{-2}, and 10^{-3} leaves crude extract concentrations on the growth of *Salmonella typhi*

Source of Variation	*SS*	*df*	*MS*	*F*	*p-value*	*F crit*
Between Groups	284.667	3	94.889	94.889	1.36E-06	4.066
Within Groups	8	8	1			
Total	292.667	11				

Discussion

This study clearly showed that the leaf crude extract of the *Cleome gynandra* can suppress *Salmonella typhi* bacteria evidenced through the formation of zones of inhibition in table 1. A decrease in the activity of the crude extract against *Salmonella typhi* was observed in the three dilutions as shown in table 1. The 10^0 exhibited the highest inhibitory effect, followed by 10^{-1} and finally 10^{-2} this may be due to decreased concentrations of active compounds in the extract and composition of extracted products. However, the positive control (ampicillin) exhibited the highest suppression. The study also coined that the crude extract could suppress bacteria as little as at a concentration of 0.15µl effectively. Analysis of variance (ANOVA) between the three concentrations (10^0, 10^{-1}, 10^{-2}) showed a statistically significant difference between the means of crude extract concentration ($p=0.05$) as in table 2. These findings are consistent with previous research that has demonstrated antimicrobial properties of *Cleome gynandra.* Ajaiyeoba, (2000) reported significant antibacterial activity against various clinical strains, which supports the results of this study. Similarly, Biswas et al., (2013) also observed considerable antimicrobial activity of *Cleome gynandra* leaf extract against multiple bacterial isolates.

Medicinal plants are considered a repository of different bioactive compounds possessing varied therapeutic properties (Raina et al., 2014). Plants are rich in a wide variety of secondary metabolites belonging to the chemical classes of tannins, saponins, terpenoids, alkaloids, and polyphenols (Khan & Kubmarawa, 2015). These represent different biological activities that depend on diversity and quantity. Therefore, from this study, it was concluded that the different phytochemicals present in the plant extracts were responsible for antibacterial activity.

Cleome gynandra contains various active phytochemicals including; Flavonoids which are important antioxidants, antiviral, anti-cancer, anti-inflammatory, and anti-allergic components (Al-Khayri et al., 2022; Mucha et al., 2021). Alkaloids are used as psychoactive substances to stimulate the nervous system and to make strong narcotic painkillers (Ganesh et al., 2018). Saponins exhibit antimicrobial properties, guarding the body against fungi, bacteria, and viruses. At the same time, they improve immune function by stimulating the production of T-cells. Additionally, they act as antioxidants and scavenge oxidative stress (Article, 2016). Since this plant has tannins exhibits anti-carcinogenic and anti-mutagenic properties mostly due to its anti-oxidizing nature. Terpenoids reduce metabolic disorders, cancer-fighting antioxidants, and anti-inflammatory agents. Phenolic compounds have antioxidant and antimicrobial properties known to exert preventive activity against infectious and degenerative diseases, inflammation, and allergies (Lokesha, 2018). Steroids work by decreasing inflammation and reducing the activity of the immune system. They are used to treat a variety of inflammatory diseases and conditions. According to a study conducted by Ganesh et al., 2018, the water extract of *Cleome gynandra* contained two active phytochemicals, which include tannins and saponins (slimy), and since the African spider plant contains these phytochemicals, it is therefore suspected that these phytochemicals are responsible for bioactivity against *Salmonella typhi*.

Conclusion

The leaves of *Cleome gynandra* can be utilized as an alternative source of useful antibiotics. The crude extracts from leaves demonstrated antibacterial activity against *Salmonella typhi*. The study also provided the rationale for the ethnobotanical application of the extracts of *Cleome gynandra* in the management of different diseases including typhoid fever. Therefore, this will solve the need to develop new antibiotics that are cheap, accessible, safe, and efficacious. While *Cleome gynandra* has demonstrated promising antibacterial properties, including against *Salmonella typhi*, it is crucial to approach its use with caution. When eaten as a vegetable, concentrated extracts might pose a risk if not properly dosed. Some individuals may be allergic to compounds in *Cleome gynandra*, leading to allergic reactions that could range from mild (rash, itching) to severe (anaphylaxis). Active compounds in the extract might interact with medications, potentially altering their efficacy or causing adverse effects. Most importantly, proper management strategies, including consultation with healthcare providers, gradual dosage introduction, and regular monitoring, are essential to minimize potential risks and ensure safe consumption.

This study used raw extract but the concentration and activity are expected to be higher when the leaves are cooked. The study reaffirms the antimicrobial potential of *Cleome gynandra*, supporting its traditional use as a medicinal plant. Continued research could lead to the development of effective natural antibacterial treatments, contributing to the diversification of therapeutic options in combating bacterial infections.

Recommendations

Since the *Cleome gynandra* has demonstrated antibacterial properties against *Salmonella typhi*, utilization of these plant leaves gradually as vegetables at a concentration of about 100-200 grams daily is recommended to suppress the infections caused by *Salmonella typhi* pathogenic bacteria. However, the African spider plant can be further exploited for developing safe and potent antimicrobials.

The current study focused on the antimicrobial activity of extracts from wet, uncooked vegetables. Future research should investigate whether these active compounds maintain their stability and efficacy at high temperatures when the vegetables are cooked. Such studies would provide valuable insights into the potential use of these compounds in food safety and preservation, as well as their applicability in culinary practices. Additionally, examining the effects of different cooking methods (e.g., boiling, steaming, frying) on the stability of these compounds would further enhance our understanding of their thermal resilience and practical applications.

Further quantitative studies are recommended to determine the efficacy of specific active phytochemicals and the precise concentrations of specified extracts for each species of *Cleome gynandra*. Such studies should employ rigorous analytical techniques, including high-performance liquid chromatography (HPLC) and mass spectrometry (MS), to identify and quantify the active compounds.

Additionally, dose-response experiments are necessary to elucidate the relationship between phytochemical concentrations and their antimicrobial effects. This research will enhance the understanding of the therapeutic potential and optimal usage of *Cleome gynandra* extracts in various applications.

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