Turkish Journal of Agriculture - Food Science and Technology, 12(s4): 2901-2906, 2024
DOI: https://doi.org/10.24925/turjaf.v12is4.2901-2906.7288

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Phytochemical Analysis and Antibacterial Effects of *Anastatica hierochuntica* Extracts on Foodborne and Clinical Pathogens

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ARTICLE INFO

Research Article

Received : 03.11.2024
Accepted : 24.11.2024

Keywords:
Phytochemical screening
Food pathogens
Clinical isolates
Agar well diffusion
Antibacterial.

ABSTRACT

This study explores the phytochemical composition and antibacterial properties of aqueous and methanolic extracts of *Anastatica hierochuntica* against foodborne pathogens and clinical isolates. Phytochemical analysis identified key compounds, including alkaloids (7.15 ± 0.0365 mg/g), flavonoids (3.16 ± 0.007 mg/g), and tannins (0.18 ± 0.0025 mg/g). Food samples yielded *Escherichia coli isolates*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella typhi*, and *Serratia marcescens*. The antibacterial efficacy was assessed using the agar well diffusion method against these pathogens and clinical isolates, including *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The aqueous extract demonstrated strong antibacterial activity against all food pathogens, with *Staphylococcus aureus*, *Salmonella typhi*, and *Serratia marcescens* exhibiting the largest inhibitory zones (34 mm). Among clinical isolates, only *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* showed selective inhibition. Conversely, the methanolic extract was largely ineffective, displaying activity only against *Serratia marcescens* (average inhibitory zone of 11.7 mm). These results underscore the potential of *A. hierochuntica* aqueous extracts as a natural antimicrobial agent and suggest further investigation into its bioactive compounds for improving food safety and addressing multidrug-resistant pathogens.

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Introduction

Foodborne illnesses remain a significant global health concern, affecting millions of individuals annually and resulting in substantial economic burdens. The World Health Organization (WHO) estimates that contaminated food causes around 600 million cases of illness and 420,000 deaths each year (Pires et al., 2024). Among the myriad of pathogens responsible for these diseases, bacteria such as *Escherichia coli*, *Salmonella spp.*, *Listeria monocytogenes*, and *Staphylococcus aureus* are particularly notorious for their prevalence and resistance to conventional antibiotics (Wu-Wu et al., 2023). The rise of antibiotic-resistant strains further complicates treatment options, prompting a renewed interest in natural antimicrobial agents derived from plants (Kiran& Venkata Mohan, 2021).

Anastatica hierochuntica, commonly known as the "resurrection plant," has garnered attention for its remarkable survival mechanisms in arid environments and its rich phytochemical composition. Traditionally utilized in various cultures for its medicinal properties, *A. hierochuntica* is reported to contain bioactive compounds such as flavonoids, alkaloids, and tannins, which are known for their antimicrobial, antioxidant, and anti-inflammatory activities (Lyubitelev & Studitsky, 2023) The plant's unique ability to revive from desiccation has led researchers to explore its potential in addressing health issues, particularly in the field of food safety (Amenu, 2014), (Kiran& Venkata Mohan, 2021).

Phytochemical profiling plays a crucial role in understanding the bioactive constituents of medicinal plants and their potential therapeutic applications. Methods such as high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS) have been employed to identify and quantify these compounds (Masoko, 2017). Understanding the chemical composition of *A. hierochuntica* could provide insights into its efficacy against foodborne pathogens, particularly in light of the increasing demand for natural preservatives and antimicrobial agents in the food industry (Badr & Quiroz 2024).

This study aims to investigate the phytochemical profile and antibacterial efficacy of both aqueous and methanolic extracts of *A. hierochuntica* against selected foodborne pathogens and clinical isolates. By employing standardized antimicrobial susceptibility testing methods, including disk diffusion and broth microdilution techniques, this research seeks to contribute to the growing body of evidence supporting the use of plant-derived extracts in food preservation and public health. The findings could not only enhance the understanding of *A. hierochuntica's* medicinal properties but also pave the way for its application in developing natural alternatives to synthetic preservatives, addressing both safety and resistance concerns in foodborne illnesses.

Given the urgency of combating foodborne pathogens and the promise of natural products in antimicrobial therapies, this investigation holds significant implications for both the scientific community and public health. It is anticipated that the results will elucidate the relationship between phytochemical composition and antibacterial activity, thereby fostering further exploration of *A. hierochuntica* as a viable candidate for enhancing food safety and health outcomes.

Materials and methods

Plant Collection and Phytochemical Analysis

To investigate the therapeutic properties of *Anastatica hierochuntica* stem, a sample was obtained from Ashifaul Haqi Prophetic Medicinal Store, located in the bustling district of Oja Oba, Ibadan, Oyo State (Latitude 7.37426º longitude 3.89513º). Following acquisition, the samples were taken to the laboratory where strict aseptic protocols were used to prevent contamination. The phytochemical screening was done to determine the bioactive constituents of the plant.

Qualitative and Quantitative Phytochemical Analysis

Phytochemical analysis for Flavonoids, Alkaloids, Glycosids, Phenolics, Saponins, Tannins were carried out using standard protocol as described by (Bakir et al., 2022)

Isolation and Characterization of Bacteria from Food Samples

In isolating pathogenic bacteria from food samples, a comprehensive microbial analysis was employed using the pour plate technique. This method was applied to isolate bacterial pathogens from a diverse array of food samples, including Rice, Salad, Suya, Zobo, and Kunu. The process began with a serial dilution of each sample, five test tubes containing 9ml each of distilled water was prepared in five places for the isolation of microorganisms from the food samples. The food samples were aseptically crushed and 1 ml of each sample was added to the 9 ml of distilled water, 1 ml from the the 4^{th} dilution from each sample was then plated was then carefully inoculated onto pre-labeled sterile plates. Selective media (Xylose Lysine Deoxycholate and Mannitol Salt Agar) were used to facilitate the growth of the pathogenic bacteria. Following culturing, the plates were incubated in an inverted position at 37°C for 24 hours, this incubation period resulted in the growth of mixed bacterial colonies. To obtain pure cultures, a series of subcultures were done. Each isolated colony was further cultured to ensure the purity of the bacterial strains. These isolates were characterized by their Macroscopically using colony morphology, growth pattern and microscopically with Gram Staining techniques and some standard biochemical characteristics which includes catalase test, citrate test, Indole and Motility test .The pure bacterial cultures were then preserved on sterile nutrient agar slants and stored in a refrigerator for future analysis and study. This methodical approach ensures that the bacterial isolates remain viable and uncontaminated for further research and potential applications.

Collection of Clinical Isolates

Pure clinical isolates of both gram-negative and gram-positive bacteria were obtained from the Medical Microbiology Laboratory at Federal Medical Centre Idi-Aba, Ogun State. These isolates were sourced from patients suffering from wounds, urinary tract, and ear infections. The bacterial isolates collected include *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa.* These isolates represent a broad spectrum of pathogenic bacteria, which are critical in understanding infection patterns and developing targeted treatment strategies.

Preparation of Aqueous and Methanolic Extracts

To prepare the plant extracts, the plant samples were reduced into smaller units to facilitate efficient drying. They were further subjected to a drying process in a hot air oven set at 50°C for 2 hours to allow for complete dryness of the wet stem according to the method described by (Abd-elmegeed et al., 2023). Following the drying process, the plant materials were pulverized into fine particles using an electric laboratory blender (LB10XS). The method of (Abd-elmegeed et al., 2023) with a little modification was used for the extraction process, three different grams of the pulverized plant material were weighed in triplicate for both extracts. For the aqueous extract, 5g of the pulverized plant material was soaked in 95 ml of distilled water, 10g in 90 ml of distilled water, and lastly 15g in 85 ml distilled water for 24hrs before it was heated up in a water bath 60°C for 5 hours. For the methanolic extract, utilizing methanol as the solvent, plant extracts were pulverized and soaked for 24hours while shaking in a shaker incubator. the mixtures were filtered through a Whatman $N_0$4 Filter Paper, and then filtrates obtained were then concentrated under reduced pressure (at 68°C) in a rotary evaporator to obtain the crude extract which kept at 4°C until further uses.

Antimicrobial Susceptibility Test

An antimicrobial susceptibility test was conducted employing the Agar well diffusion method to evaluate the effectiveness of the extracts against the isolated bacteria. This testing procedure involved several key steps to ensure accurate and reliable results. Mueller Hinton agar was prepared following the manufacturer's instructions. The molten agar was aseptically poured into pre-labeled plates and allowed to solidify, creating a uniform medium for bacterial growth. Following agar preparation, a sterile swab stick was used to evenly inoculate the surface of the agar plates with the bacterial culture. This ensured a consistent bacterial lawn across the agar surface, essential for evaluating antimicrobial activity. Wells of approximately 3-4 mm in diameter were created on the

agar plates using a sterile cork borer. Using a sterile syringe, 0.5 ml of different concentrations of aqueous and methanolic extracts were dispensed in the wells. Each well contained a distinct concentration of the extract, allowing for a comparative analysis of antimicrobial efficacy. The plates were incubated at 37°C for 24 hours to allow for bacterial growth and the diffusion of antimicrobial agents from the wells. After the incubation period, the presence of clear zones around each well was observed. These clear zones indicated areas where bacterial growth had been inhibited by the antimicrobial agents in the extracts. The diameter of each zone of inhibition was measured in millimeters (mm) using a sterile measuring ruler. This measurement provided quantitative data on the effectiveness of the different extracts in inhibiting bacterial growth.

Statistical Analysis

Data obtained were subjected to statistical analysis using the Statistical Package for Social Sciences (SPSS) version 20.0. Mean values were compared using Analysis of Variance (ANOVA). Results were presented as Mean±Standard deviation. A post hoc test was done using the Student-Newman-Keuls (SNK) to compare mean values between the treatment groups. A probability value (p-value) less than 0.05 was considered to be statistically significant.

Results

The phytochemical profile of *Anastatica hierochuntica* and their mean occurrence is presented in Table 1 and Figure 1 respectively. The study involved a meticulous screening of the plant to identify its bioactive compounds, which included a diverse array of anti-therapeutics.

Table 2 shows the biochemical characterization of bacteria isolated from herbs including Gram Reaction, Catalase, Citrate, Indole, and Motility.

Antibacterial Activities of Anastatica hierochuntica Extracts on Food Pathogens

Table 3 shows the antibacterial activities of the extract of *A. hierochuntica* against five pathogenic bacteria isolated from food samples: *Escherichia coli*, *Salmonella typhi*, *Serratia marcescens*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*.

The aqueous extract of the plant exhibited broad-spectrum antibacterial effects on *Salmonella typhi*, *Serratia marcescens*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* across all tested concentrations. *Escherichia coli*, however, only showed susceptibility at a concentration of 10g/90ml, with an inhibitory zone of 10mm.

Table 1. Phytochemical Analysis of *Anastatica hierochuntica*

	Qualitative		Quantitative
	(a)	(b)	(Mean ± SEM)
Tannin	+	+	0.1835 ± 0.0025
Saponin	++	++	1.3465 ± 0.0175
Flavonoid	+	+	3.1550 ± 0.0070
Alkaloids	++	++	7.1485 ± 0.0365
Phenol	+	+	0.3690 ± 0.0120
Glycoside	+	+	0.1140 ± 0.0020
Phytate	++	++	0.7785 ± 0.0935
Steroid	+	+	0.0905 ± 0.0055
Anthraquinone	++	++	0.9850 ± 0.0030
Anthocyanin	+	+	0.1055 ± 0.0035

+ present; ++ highly present

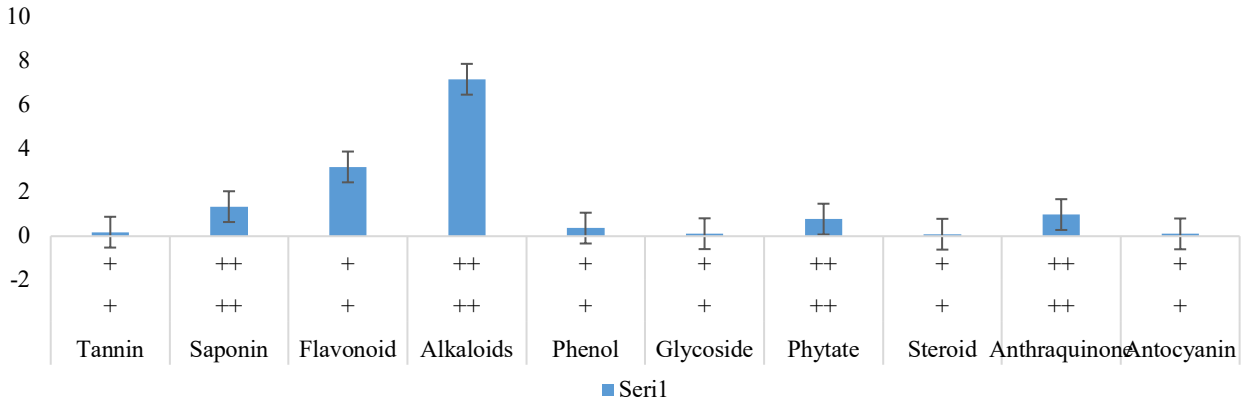

Figure 1. Phytochemical Profile of *Anastatica hierochuntica*

Table 2. Biochemical characterization of Food Borne Isolate

Organisms	Gram staining	Shape	Catalase	Citrate	Indole	Motility
Escherichia coli	_	Rod	+	_	+	Motile
Staphylococcus aureus	+	Cocci	+	+	_	Non motile
Staphylococcus epidemidis	+	Cocci	+	_	_	Non motile
Salmonella typhi	_	Rod	+	+	_	Motile
Serratia marcescens	_	Rod	+	+	_	Motile

Table 3. Zone of inhibition (mm) of *A.hierochuntica* against Food pathogens

Extracts	*Escherichia coli*	*Staphylococcus aureus*	*Staphylococcus epidemidis*	*Salmonella typhi*	*Serratia marcescens*
A (5g/95ml)	-	24± 0.31	13± 0.10	17± 0.00	34± 0.41
A(10g/90ml)	10± 0.00	15± 0.50	20± 0.70	15± 0.20	32± 0.60
A(15g/85ml)	-	34± 0.80	24± 0.70	34± 0.00	17± 0.00
M (5g/95ml)	-	-	-	-	10± 0.00
M(10g/90ml)	-	-	-	-	15± 0.00
M(15g/85ml)	-	-	-	-	10± 0.00

No inhibition zone with the use of Methanol at all concentrations for *E.coli, S.aureus , S. epidemidis* and *S. typhi.* Significant inhibition changes in aqueous extracts across all organisms concentrations increased was observed. (p ≥0.05); A: Aqueous Extract; M: Methanol Extract

A B

Plate 1. A: Zones of Inhibition of Aqueous extract of *A.hierochuntica* stem on , *S.aureus* , *S. epidemidis* and *S. typhi* B: *Serratia marcescens*

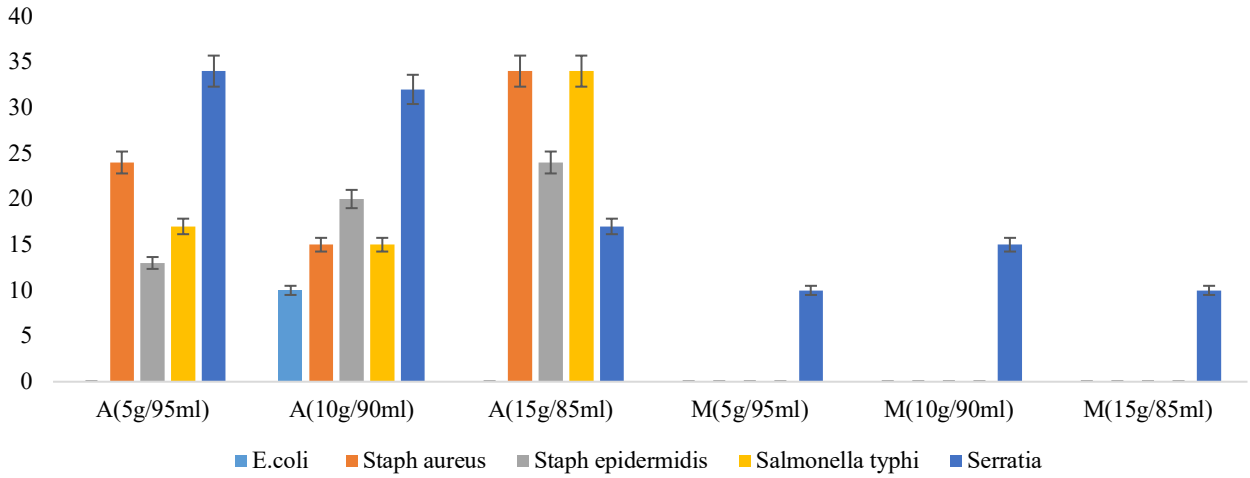

Figure 3. Comparative mean of Aqueous and Methanolic extracts of *Anastatica hierochuntica* on isolated food pathogens.

The methanolic extract demonstrated limited effectiveness against the tested bacteria, showing notable activity only against *Serratia marcescens*. The methanolic extract produced inhibitory zones of 10mm, 15mm, and 1mm at different concentrations as shown in Figure 3.

Antibacterial activity of Anastatica hierochuntica Extracts on Clinical Isolates

The effects of aqueous extract of *Anastatica hierochuntica* demonstrated selective antibacterial activity with significant effects against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, but not on *Staphylococcus aureus* and *Escherichia coli*, as shown in Table 4. Methanolic extract of *Anastatica hierochuntica* did not exhibit any measurable antibacterial activity against the tested bacterial isolates.

Discussion

Medicinal plants have a long therapeutic history and are currently regarded as a promising source of medicine in the conventional healthcare system (Saleh et al.,2021). The findings of this study provide compelling evidence for the phytochemical richness and antibacterial potential of *Anastatica hierochuntica* extracts, particularly the aqueous variant, against various foodborne pathogens and clinical isolates.

Table 4. Zone of inhibition (mm) of *A.hierochuntica* on selected clinical isolates.

Extracts	*Klebsiella pneumoniae*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*
Aqueous(5g/95ml)	34± 0.40	-	10± 0.00	-
Aqueous(10g/90ml)	15± 0.00	-	22± 0.00	-
Aqueous(15g/85ml)	13± 0.00	-	15± 0.00	-
Methanol(5g/95ml)	-	-	-	-
Methanol(10g/90ml)	-	-	-	-
Methanol(15g/85ml)	-	-	-	-

No inhibition zones was observed for all concentrations with Methanolic extract, Aqueous extract of the stem was only able to inhibit a low concentrations ($p \geq 0.05$); A: Aqueous Extract; M: Methanol Extract

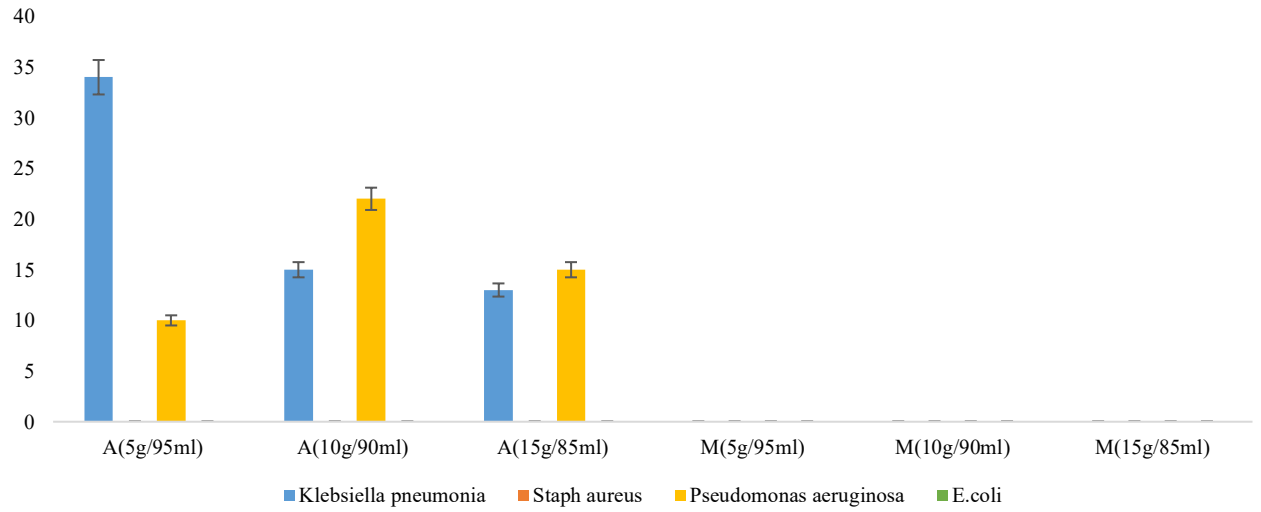

Figure 4. Mean antibacterial susceptibility of *Anastatica hierochuntica* extracts against selected clinical isolates

The detected phytochemicals, alkaloids, flavonoids, and tannins are known for their diverse biological activities, including antimicrobial properties as reported in the study of (Tavares & Seca, 2018). The quantification of phytochemicals revealed the presence of alkaloids (7.15 ± 0.0365 mg/g), flavonoids (3.16 ± 0.007 mg/g), and tannins (0.18 ± 0.0025 mg/g) in *A. hierochuntica* extracts. Alkaloids are recognized for their ability to disrupt microbial cell functions and interfere with nucleic acid synthesis (Sathiyaseelan et al., 2020). Flavonoids possess strong antioxidant properties and contribute to the inhibition of bacterial growth by disrupting cell membrane integrity and function (Pareek et al., 2023) Tannins, though present in lower concentrations, are also known for their antimicrobial effects, particularly through protein precipitation and enzyme inhibition (Sathiyaseelan et al., 2020). The synergy of these compounds likely contributes to the robust antibacterial activity observed in the aqueous extracts. The results indicate that the aqueous extract of *A. hierochuntica* exhibits strong antibacterial activity against a range of foodborne pathogens, with significant inhibitory zones measured, particularly against *Staphylococcus aureus*, *Salmonella typhi*, and *Serratia marcescens* (34 mm). This aligns with the research of (Guo et al., 2017). highlighting the effectiveness of plant extracts against these pathogens, which are common culprits in foodborne illnesses. The broad-spectrum activity against foodborne pathogens suggests that *A. hierochuntica* could serve as a potential natural preservative in food systems, thereby enhancing food safety while minimizing reliance on synthetic additives. In contrast, the methanolic extract in this study demonstrated limited antibacterial efficacy, showing activity solely against *Serratia marcescens*. This disparity could be attributed to the solubility of certain phytochemicals; the polar nature of aqueous solvents may better extract hydrophilic compounds with potent antibacterial properties, while methanolic extraction may favor less effective compounds (Quinto et al., 2019). The findings underscore the importance of solvent choice in phytochemical extraction for maximizing biological activity. The selective inhibition observed against clinical isolates, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*—suggests potential therapeutic applications in clinical settings, particularly as these organisms are increasingly recognized for their multidrug-resistant characteristics (Ihsanullah, 2012). The ability of *A. hierochuntica* to inhibit these pathogens raises questions about the underlying mechanisms, which could involve disruption of membrane integrity or interference with metabolic pathways due to the identified phytochemicals. Furthermore, the findings from this study highlight the potential of *A. hierochuntica* as a candidate for natural antimicrobial development, especially in an era marked by rising antibiotic resistance. The increasing prevalence of multidrug-resistant pathogens necessitates innovative solutions, and plant-based antimicrobials could play a crucial role (Bhattacharjee & Islam 2015). Further research into the isolation and characterization of individual bioactive compounds within *A. hierochuntica* could elucidate specific mechanisms of action and facilitate the development of effective natural antimicrobials.

Future studies should focus on expanding the range of pathogens tested and exploring the potential synergistic effects of the phytochemicals identified in *A. hierochuntica*. Additionally, in vivo studies are warranted to assess the efficacy and safety of these extracts in real-world applications, such as food preservation and therapeutic use.

Conclusion

In conclusion, this study highlights the promising antibacterial properties of aqueous extracts of *Anastatica hierochuntica,* demonstrating significant effectiveness against a range of foodborne pathogens and clinical isolates. The presence of key phytochemicals, such as alkaloids, flavonoids, and tannins, may contribute to the observed antimicrobial activity, particularly against strains like *Staphylococcus aureus* and *Salmonella typhi*. In contrast, the methanolic extracts showed limited efficacy, indicating that the extraction method plays a crucial role in the bioactivity of the compounds. These findings not only advocate for the use of *A. hierochuntica* as a natural antimicrobial agent but also pave the way for future research into its bioactive components. Such investigations could enhance food safety measures and provide alternative strategies for combating multidrug-resistant pathogens.

Declarations

This study was presented at the 7th International Anatolian Agriculture, Food, Environment and Biology Congress, (Kastamonu, TARGID 2024)

Author contribution statement

Amina Badmos: Project administration, supervision, conceptualization, methodology, review and editing. Onikola Hanahu and Sekina Onigbinde and Onikola Rasaq: Data collection, Investigation, Formal analysis, writing the original draft.

Fund Statement

No funding was received for this research

Conflict of Interest

The authors declare no conflict of interest

Acknowledgment

The authors acknowledges the staff of the Microbiology Laboratory of the Federal University of Agriculture Abeokuta for the space and some equipments used during the course of the experiment.

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