Herbal Antimicrobials in Perspective: Evaluating *Andrographis paniculata* (Acanthaceae) against Vaginal *Staphylococcus aureus*

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Abstract: The rising antimicrobial resistance burden constitutes a paramount worldwide health threat, particularly in low-resource environments. *Staphylococcus aureus*, inclusive of methicillin-resistant *S. aureus* (MRSA), is a common cause of vaginal infection among reproductive-aged women. The present study evaluated the antimicrobial property of ethanolic vinegar leaf (*Andrographis paniculata*.) extract against *S. aureus* isolates from high vaginal swabs (HVS) of patients attending Madonna University Teaching Hospital, Elele, Nigeria. A total of 30 clinical isolates of *S. aureus* were identified using standard microbiological techniques. Vinegar leaves were processed and extracted using ethanol, and antimicrobial activity was tested via the disc diffusion method at concentrations of 100%, 75%, and 50%. Results revealed the highest prevalence of S. aureus among the age group 20–30 years (40%). The extract showed concentration-dependent antibacterial activity with the highest sensitivity at 100% concentration across all age groups. Specifically, 91.7% of isolates of the 20–30 year age group were sensitive at 100%, and the sensitivity decreased considerably at lower concentrations. Although sensitivity and resistance patterns varied among age groups and concentrations, the chi-square test indicated the absence of a statistically significant association ($p > 0.05$). These findings suggest that vinegar leaf extract possesses promising antimicrobial activity, particularly at higher concentrations, and may serve as an adjunct therapy in the treatment of S. aureus-related infections. Further studies with larger samples and phytochemical profiling are warranted to elucidate active principles and standardise therapeutic use.

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1. Introduction

The emerging issue of antimicrobial resistance (AMR) has also become a significant global health concern, particularly in low- and middle-income countries. *Staphylococcus aureus*, including methicillin-resistant strains

(MRSA), is among the major pathogens implicated in a variety of infections, including female reproductive tract infections. Recent studies have reported high prevalence of vaginal colonization by antimicrobial-resistant bacteria among women in labor, highlighting the potential risk to both maternal and neonatal [1]

The increasing resistance of *S. aureus* to conventional antibiotics necessitates the quest for alternative therapeutic agents. Plant compounds have been of interest due to their diverse bioactive constituents and extensive history of utilisation in traditional medicine. Ethanolic plant extracts have been found to exhibit significant antimicrobial activity against *S. aureus*. For instance, studies have reported that ethanolic plant extracts like those of *Syzygium antisepticum* and *Acacia nilotica* possess potent antibacterial activity against *S. aureus*, based on their rich phytochemical profiles [2-4].

"Vinegar leaf," as commonly referred to in certain regions, has traditionally been exploited for medicinal properties, including antimicrobial activity [5]. While no particular scientific studies on vinegar leaf exist, its application in traditional medicine suggests the potential presence of bioactive compounds that could be inhibitory to pathogenic bacteria.

Investigating the antimicrobial activity of its ethanolic extract against *S. aureus* isolated from high vaginal samples can provide insights into alternative treatment options, especially for regions with limited access to conventional antibiotics. This study is aimed at evaluating the antimicrobial activity of the ethanolic extract of vinegar leaf against *Staphylococcus aureus* strains isolated from high vaginal swabs of patients in Madonna University Teaching Hospital. The finding may contribute to the development of plant-based antimicrobials as an adjunct in the treatment of infections caused by resistant strains of bacteria.

2. Materials and Methods

2.1. Study Area

The research was carried out at Madonna University Teaching Hospital (MUTH), Elele, Rivers State, Nigeria. The hospital is a tertiary health facility and a teaching and research hospital of Madonna University.

2.2. Authentication of the Leaf sample

The botanical identity of the leaf sample was confirmed by Mr. Ogunlowo I. I. at the Medicinal Plants Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife (Code: FPI), included in the online edition of Index Herbariorum available on: https://sweetgum.nybg.org/science/ih/herbarium-details/?irn=253433.
***Andrographis paniculata* (Burm.f.) Wall. ex Nees. Family:** Acanthaceae. **Voucher No.:** FPI 2646

2.3. Collection and Preparation of Vinegar Leaf Extract

Fresh vinegar leaves (***Andrographis paniculata***) were harvested from a market in Elele, Rivers State, Nigeria. Ethanol extracts of the leaves were prepared as per the procedure of [6-11]. The leaves were cleaned in clear water extensively, oven-dried at 45 °C for 12 hours and ground. After grinding, it was turned into a 2000ml conical flask, and a total of 42000ml of methanol solution was poured into the conical flask containing the powder form. it was stirred properly and covered with foil paper. The maceration lasted for 72 hours and was stirred for a 24-hour interval. It was then filtered using a Whatman filter paper, and the filtrate was poured into a beaker (1000 mL). The filtrate was placed in the water bath for evaporation. The filtrate was turned into dried extract and was weighed using an electronic weighing balance. The extract possesses a final weight of 2.6 g. The extract was maintained in tightly closed containers at 4 °C. Working concentrations (100%, 75%, and 50%) of distilled water were prepared for antimicrobial testing.

2.4. Isolation and Identification of *Staphylococcus aureus*

Thirty (30) clinical isolates of *Staphylococcus aureus* were obtained from high vaginal swab (HVS) specimens of patients attending MUTH. The swabs were plated on Mannitol Salt Agar (MSA), a selective and differential medium for *S. aureus*, and incubated at 37 °C for 24 hours [12**]**. Colonies showing typical yellow fermentation on MSA were subjected to morphological and biochemical identification in the form of Gram staining, catalase, and coagulase tests [13**]**.

2.5. Antimicrobial Susceptibility Testing

The antimicrobial activity of the ethanolic vinegar leaf extract was evaluated using the modified Kirby-Bauer disc diffusion method, following Clinical and Laboratory Standards Institute (CLSI) guidelines [14]. Filter paper discs of 6 mm diameter were prepared from Whatman No. 1 filter paper and autoclaved at 160 °C for 1 h to sterilise them. The

discs were filled with either extract concentrations (100%, 75%, 50%), allowed to stay at room temperature under air flow, and aseptically stored. Mueller-Hinton Agar (MHA) plates were seeded with standardised *S. aureus* suspension (0.5 McFarland standard; ~1.5×10^8 CFU/mL) through sterile swabs. Discs impregnated with the extracts were placed in a uniform pattern onto the agar surface and left to pre-diffuse for 1 hour at room temperature. Plates were incubated at 37 °C for 24 hours. The zone of inhibition was quantified in millimetres and recorded [14].

2.6. Data Analysis

The data were evaluated for the determination of the distribution of *Staphylococcus aureus* isolates from high vaginal swabs (HVS) among various age groups and susceptibility to ethanol extract of vinegar leaves at various concentrations (100%, 75%, and 50%) among MUTH patients. The percentage occurrence of *S. aureus* isolates by each age group was determined using descriptive statistics. Susceptibility and resistance patterns were reported as frequencies and percentages. To assess the correlation of age groups with susceptibility or resistance to different concentrations of ethanolic extract, the Chi-square test (χ^2) was employed. $p < 0.05$ was maintained as a statistical criterion of significance.

3. Results

Table 1 depicts the occurrence of *S. aureus* isolates by age groups. Thirty isolates were detected in HVS samples. The highest occurrence was detected in the 20–30 years group (40%), followed by 31–40 years (33.3%) and 41–50 years (26.7%).

Table 1: Occurrence of *Staphylococcus aureus* in HVS by Age Group

Age Group (years)	Number of Isolates	Percentage (%)
>20	—	—
20–30	12	40
31–40	10	33.3
41–50	8	26.7
Total	**30**	**100**

The susceptibility of *S. aureus* isolates to the ethanol extract of vinegar leaves at concentrations of 100%, 75%, and 50% was evaluated (Table 2). The results showed variable sensitivity and resistance across age groups and extract concentrations.

Table 2: Susceptibility of *Staphylococcus aureus* to Ethanol Extract of Vinegar Leaves by Age Group

Age Group	Concentration	Sensitive (n)	Resistant (n)
20–30	100%	11	1
	75%	8	4
	50%	2	10
31–40	100%	7	3
	75%	5	5
	50%	1	9
41–50	100%	6	2
	75%	4	4
	50%	3	5

Chi-square test revealed no significant relationship between age groups and resistance or susceptibility at any concentration ($p > 0.05$), even where the frequency of resistance and sensitivity differed. The reported p-values for sensitivity (0.044) and resistance (0.013) were above threshold when the chi-square values ($X^2 = 2.498$ and 0.287 respectively) were considered, indicating no significant difference.

Figure 1 shows the percentage susceptibility and resistance overall of *S. aureus* isolates to ethanolic extract at different concentrations. The extract was most sensitive at 100% concentration, with a profound decrease in sensitivity with decreasing concentration.

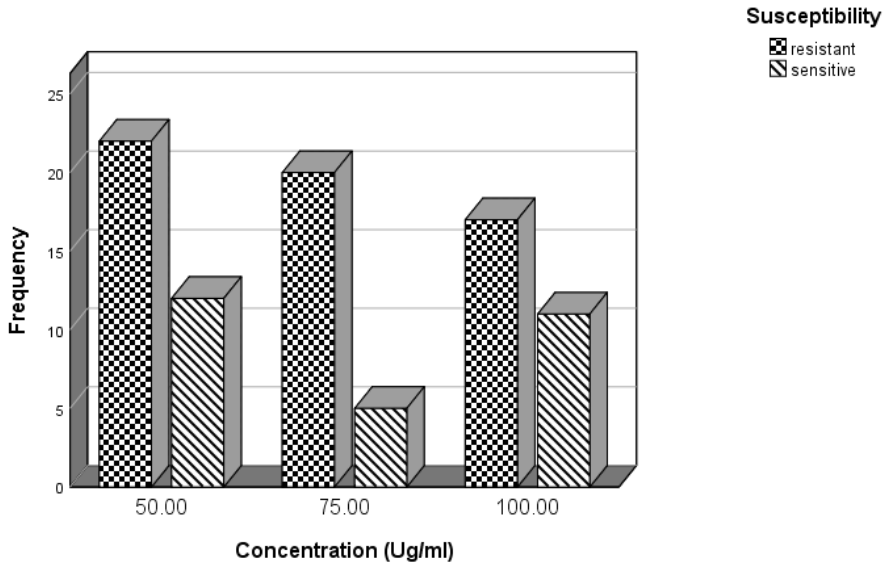

Figure 1: Percentage susceptibility and resistance of *Staphylococcus aureus* to ethanol extract of vinegar

4. Discussions

Antimicrobial resistance (AMR) development has increasingly cut short the effectiveness of conventional antibiotics, with renewed attention to other drugs, especially those from medicinal plants. In this study, the in vitro antimicrobial activity of ethanolic extract of *Andrographis paniculata* (also referred to as vinegar leaf) against clinical isolates of *Staphylococcus aureus* from high vaginal swabs of patients in Madonna University Teaching Hospital, Elele, Nigeria was determined.

Prevalence of *S. aureus* from vaginal swabs was highest among women aged 20–30 years (40%), a finding that is consistent with results in similar studies of increased colonization in sexually active reproductive-aged women [1]. Colonization at this age may be attributed to hormonal fluctuations, sexual behavior, and intravaginal product use, which compromise the vaginal microbiota and make individuals susceptible to pathogenic microbes [15]. This trend also aligns with previous reports of higher colonisation rates among women in their reproductive years, which could be attributed to high sexual activity, endogenous hormonal fluctuations, and frequent medical interventions such as pelvic examinations or antibiotic treatment [16]. Notably, a single study conducted in Uganda reported a colonisation rate of 8.2% among women in labour, indicating the need for monitoring of S. aureus colonisation in this population [17].

A. paniculata ethanolic extract exhibited concentration-dependent antibacterial activity against S. aureus isolates. The extract, at 100% concentration, recorded high antimicrobial activity with susceptibility in 80–91.7% of isolates across all age groups. This finding is consistent with previous reports that have demonstrated high antibacterial activity of *A. paniculata* extracts against Gram-positive bacteria, including *S. aureus* [4; 10; 18]. The decreased susceptibility at 75% and 50% concentrations vindicates the necessity of dosage optimisation in phytotherapeutic application. These findings are consistent with previous research that the antimicrobial effectiveness of plant extracts is generally concentration-dependent and also with the presence of phytochemical constituents such as tannins, flavonoids, and phenolic acids [19].

The reported bioactivity may be due to the presence of phytochemicals such as flavonoids, diterpenoids (e.g., andrographolide), and alkaloids, reported to disrupt bacterial cell membrane, inhibit DNA synthesis, and hinder protein metabolism [2; 5]. Without thorough phytochemical screening, however, the specific active components responsible for this reported antibacterial activity remain speculative.

Despite variations in susceptibility by age group and concentration, the statistical analysis revealed no significant association ($p > 0.05$), which is a sign that host-related variables like age can possibly not impact the efficacy of the extract significantly. Non-significant correlations between demographic variables and microbial susceptibility are indicated by similar studies employing herbal extracts [3].

Interestingly, the antimicrobial activity of the *A. paniculata* extract indicates its potential as an adjunct or alternative therapy for the management of vaginal infections, especially in regions with high antimicrobial resistance and limited

access to contemporary therapeutics. With traditional use established and demonstrated safety, *A. paniculata* is a candidate for the development of topical formulations or oral medications against *S. aureus* infections.
Still, this study has a number of limitations. It had a small sample size (n = 30), and there was no comparator with a standard antibiotic for comparative efficacy. Also, the phytochemical profile of the extract was not examined, nor was its toxicology. Future studies should focus on profiling the active molecules using chromatographic and spectrometric methods, determining cytotoxicity, and conducting in vivo tests to confirm safety and efficacy.

5. Conclusion

The current research demonstrates that ethanolic extract of *Andrographis paniculata* possesses potential antibacterial activity against *Staphylococcus aureus* isolated from high vaginal swabs, particularly at higher doses (100%). Antimicrobial activity depending on the concentration makes it a strong candidate as a complementary therapy in the treatment of vaginal *S. aureus* infections, most notably in view of the growing antimicrobial resistance. However, to determine its therapeutic potential, its bioactive constituents must be elucidated, as well as dosages, through additional studies, and its pharmacokinetics and safety in clinical situations must be established.

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