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Susceptibility Patterns of *Staphylococcus aureus* Isolated from Wounds Swabs to Extracts of *Vernonia amygdalina*

O. Oluyele[1*] and M. K.Oladunmoye[1]

[1]Department of Microbiology, The Federal University of Technology, Akure, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author MKO designed the study and wrote the protocol. Author OO performed the statistical analysis, wrote the first draft of the manuscript, managed the analyses of the study and managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: There is an ever growing interest in investigating different groups of plants to identify their potential therapeutic applications. This is due to a tremendous historical legacy in folk medicine use of plants as remedy for treating diseases. *Vernonia amygdalina* have been shown to exhibit profound ethnomedical and pharmacological properties. The present study investigated the susceptibility patterns of *Staphylococcus aureus* isolated from wound infections to extracts of *Vernonia amygdalina.*
Study Design: This study was designed to investigate the susceptibility patterns of *Staphylococcus aureus* isolated from wound sites of patients attending four hospitals in Akure to extracts of *Vernonia amygdalina*.
Place and Duration of Study: This research was carried out in the Department of Microbiology Federal University of Technology Akure, between November 2015 and April 2016.
Methodology: Fresh leaves of *Vernonia amygdalina* were collected between November 2015 and January 2016 from a farm in Akure and identified at the Department of Crop, Soil and Pest

**Corresponding author: E-mail: olumideoluyele@gmail.com;*

Management, Federal University Technology, Akure (FUTA). The leaf extraction was carried out using four solvents (60% ethanol, cold water, hot water and chloroform). Agar well diffusion technique was used for susceptibility testing of isolates.

Results: The phytochemical screening of the extracts revealed various constituents which includes: flavonoids, alkaloids, terpenoids, phenols, cardiac glycoside, saponins and tannins. The ethanol extract showed the lowest Minimum Inhibitory Concentration value (12.5 mg/ml), while the chloroform extract showed the lowest zone of inhibition (10.33 mm) against the *S. aureus* isolates at 100 mg/ml. The ethanol extract of the plant showed the highest potency against the tested isolates, while the chloroform extract showed the lowest efficacy.

Conclusion: The results of this study suggests that *Vernonia amygdalina* leaf extracts can be used as potential herbs for drug development for the treatment of infections caused by *Staphylococcus aureus*. This may also help reduce the overdependence on commercial antibiotics and cases of antibiotics resistance.

Keywords: Vernonia amygdalina; Staphylococcus aureus; phytochemicals.

1. INTRODUCTION

Staphylococcus aureus is notorious for its ability to become resistant to antibiotics [1,2]. Infections due to antibiotics resistant strains of *S. aureus* are associated with increased burden on health resources; and increased morbidity and mortality [3-5].

Based on ethnomedical practices and coupled with the fact that numerous infectious agents are becoming resistant to synthetic drugs, researchers are focusing on the discovery of new therapeutic substances of natural origin with possible low or no toxicity to human, animal and environment [6]. *Vernonia amygdalina* is a valuable medicinal plant widespread in West Africa and reputed to have several health benefits [7]. It is used either alone or incorporated as an important ingredient in traditional polyherbal formulations to treat various diseases [8-10].

Nutritionally, the macerated leaves of the plant is used mainly in soup making in the tropics and also as an appetizer and febrifuge [11] and has been successfully used as a supplement in weaning foods [12]. *V. amygdalina* has been found to be rich in minerals, especially phosphorus, calcium, potassium, magnesium, zinc, iron and some vitamins like vitamin A, C and E [13]. *V. amygdalina* extracts have been shown to exhibit profound ethnomedical and pharmacological properties viz: anti-diabetic [14], anti-tumorigenic properties [15,16], anti-plasmodial [17], anti-helminthic [18] and anti-bacterial properties [7] The present study investigated the susceptibility patterns of *Staphylococcus aureus* isolated from wound infections to extracts of *Vernonia amygdalina.*

2. MATERIALS AND METHODS

2.1 Isolation and Identification of *Staphylococcus aureus*

Staphylococcus aureus was recovered from different wound types (accident wounds, post-operative wounds, burns and vaginal delivery wounds) of patients with wound infections attending four hospitals in Akure, Ondo State. Preliminary identification of the isolates was based on Gram staining reaction, morphology and cultural characteristics on Manitol Salt Agar (MSA). Furthermore, characterization was carried out using various biochemical tests which include: catalase test, coagulase test, indole production test, citrate utilization test, methyl- red test, voges-proskauer test and sugar fermentation tests as described by [19].

2.2 Source of Typed Isolate

Staphylococcus aureus ATCC 25923 was obtained from the Nigerian Institute of Medical Research (NIMR) Lagos.

2.3 Ethical Approval

Ethical approval for the study was obtained from the Ethics and Research Section of the Ondo State Ministry of Health.

2.4 Collection and Identification of Plant Materials

Fresh leaves of *Vernonia amygdalina* were collected from a farm in Akure, Ondo State between November 2015 and January 2016 and identified at the Crop, Soil and Pest Management Department in Federal University of Technology, Akure (FUTA).

2.5 Preparation of Leaf Extracts of *Vernonia amygdalina* and Extraction of Plant Materials

The fresh leaves were air dried at room temperature for four weeks until fully crispy. The dried leaves were ground into powdered form using mortar and pestle. They were kept in an airtight container to avoid the absorption of moisture. Two hundred grammes (200 g) of the powdered leaves were soaked in 1200 ml of the extraction solvents (60% ethanol, cold water, hot water and chloroform) separately for 72 hours with occasional shaking. The mixtures were sieved using muslin cloth and then filtered using milipore filter paper (0.45 microns). The filtrates were concentrated using rotary evaporator. The extracts obtained were stored in the refrigerator at 20℃ for further studies.

2.6 Sterility Proofing of the Extract

The extracts were tested for sterility after Millipore filtration by introducing 2 ml of the sterile extract into 10 ml of sterile Mueller Hilton broth. This was incubated at 37℃ for 24 hours. A sterile extract was indicated by absence of turbidity or clearness of the broth after the incubation period [20].

2.7 Qualitative Phytochemical Analysis of *Vernonia amygdalina*

Qualitative and quantitative phytochemical analyses were carried out on the extracts using standard chemical methods as described by [21].

2.8 Susceptibility Testing of the Isolates to Plant Extracts

Agar well diffusion technique was used [19] to determine the antibacterial activity of plant extracts. Mueller Hinton agar plates were prepared following the manufacturer's instructions. Zero point one (0.1) ml aliquot of each test organism suspension (standardized) was transferred onto the well dried Mueller Hinton agar plates in triplicate and was spread evenly using sterile swab sticks. The plates were allowed to dry, a standard sterile cork borer of 8mm diameter was used to cut uniform wells on the Mueller Hinton agar plates and the centre well served as the control. Each well was appropriately labeled on the reverse side of the plates. A sterile pipette was used to transfer 0.2 ml of the plant extracts into respective wells in the agar plates. Sterile water was used as negative control in the centre well, while Ofloxacin (10 µg) or Vancomycin (30 µg) was used as positive control in one of the wells. The plates were allowed to stand for 30 minutes at room temperature to allow proper diffusion of the extract to occur. All the plates were incubated at 37℃ for 24 hours, after which, the zones of inhibition were measured using transparent meter ruler to the nearest millimeters.

2.9 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC and MBC of the extract was determined using dilution method [19]. Different concentrations of the crude extracts were prepared at 400, 200, 100, 50, 25, 12.5 and 6.25 mg/ml. Mueller Hinton broth was prepared and 5 ml was dispensed into the test-tubes containing varying concentrations of the extracts. This was followed by the addition of 0.1 ml of *S. aureus* inoculum (1 x 10^6cell/ml) into each test tube. A set of test tubes containing only Mueller Hilton broth were used as negative control, another set of test tubes containing Mueller Hilton broth and test organisms were used as positive control. All the test tubes were then incubated at 37℃ for 24 hours. Growth in each tube was checked for by using a spectrophotometer (Beckman model 35). Growth inhibition was indicated by no/low turbidity while growth was indicated by high turbidity. The concentration at which there was no/least growth as indicated by clear broth is taken as the MIC. The MBC was determined by taking a loopful from each tube that showed no growth during MIC determination and streaked onto Mueller Hilton agar plates and incubated at 37℃ for 24 hours. The least concentration at which no growth was observed was noted as the MBC.

3. RESULTS AND DISCUSSION

3.1 Percentage Recovery of *Vernonia amygdalina*

Although cold water, hot water and chloroform as solvents had extracting capabilities, ethanol extract of *V. amygdalina* had the highest percentage yield. The varying percentage yields obtained in this study may be due to the various solvents used for extraction as suggested by [22], who reported that polar solvents have been shown to be more effective in extracting organic

and inorganic materials from plants. Ethanol which is classified as a polar protic solvent has a slightly low dipole and dielectric constant, as such; it has the ability to extract multivariable compounds from plants [23]. Also, according to [24] nearly all identified compounds from plants active against microorganisms are aromatic or saturated organic compounds, they are most often obtained through ethanol or methanol extraction. Thus, from the results it is evident that the intracellular constituents from the plants are miscible in ethanol giving it a higher percentage yield than other solvents employed in this study. Chloroform gave the least percentage yield probably because it is a less polar solvent and has affinity for lipophilic substances as such may be unable to extract the hydrophilic components of the plant [25].

3.2 Qualitative Phytochemical Composition of *Vernonia amygdalina* Extracts

The result of the qualitative phytochemical analysis of the extracts revealed the presence of flavonoid, alkaloid, saponin, tannin, terpenoid, cardiacglycosides and phenols. This result is presented in Table 2.

The qualitative phytochemical analysis of the various extracts of *V. amygdalina* demonstrated the presence of tannins, saponins, flavonoids, terpenoids, steroids, cardiac glycosides, alkaloids and phenols. This is in agreement with the work of [25] which showed the presence of these phytochemical constituents in the stem and root bark extracts of *V. amygdalina*. The result is further in consonance with the work of [20] which showed that the same phytochemical constituents are present in the leaf extracts of the plant. These phytochemicals have been shown to exhibit various pharmacological and biochemical actions and are probably responsible for the antibacterial activity of the plant [26].

3.3 Quantitative Phytochemical Composition of *Vernonia amygdalina* Extracts

Comparatively, the quantitative phytochemical analysis of the extracts showed that ethanol extract of the plant had the highest values for flavonoids (12.30 ± 0.10 mg/g) and tannins (5.23 ± 0.84 mg/g); cold water extract had the highest value for phenols (9.77 ± 0.13 mg/g) and saponin (5.09 ± 0.07 mg/g); hot water extract had the highest value for alkaloids; while chloroform extract had the lowest values for alkaloids (8.23 ± 1.79 mg/g), flavonoids (5.10 ± 0.12 mg/g) and saponin. The result is presented in Fig. 1.

The observed quantitative distribution of the various phytochemicals in the extracts could be due to their relative solubility in the various solvents used for extraction. According to Srinivasav et al. (2001) different solvents have different spectrum of solubility for phytoconstituents.

Table 1. Percentage recovery of *Vernonia amygdalina*

Solvent	Original weight (g)	Extracted weight (g)	Percentage recovery (%)
Ethanol	200	21.1	10.55
Cold water	200	17.80	8.90
Hot water	200	17.10	8.55
Chloroform	200	9.60	4.80

Table 2. Qualitative phytochemical composition of *Vernonia amygdalina* extracts

Phytochemicals	Extracts			
	Ethanol	Cold water	Hot water	Chloroform
Tannins	+	+	+	+
Saponins	+	+	+	+
Flavonoids	+	+	+	+
Terpenoids	+	-	+	-
Cardiac-glycoside	+	+	+	+
Alkaloids	+	+	+	+
Phenols	+	+	+	+

Legend : + = present, - = absent

3.4 Mean Zones of Inhibition of *Staphylococcus aureus* Isolates from Wounds to Extracts of *Vernonia amygdalina* at 100 mg/ml

The results of the mean zones of inhibition of *Vernonia amygdalina* extracts at 100mg/ml to *Staphylococcus aureus* isolates from wounds are presented in Figs. 2a, 2b, 2c, 2d and 2e. The ethanolic extract of *Vernonia amygdalina* gave the highest mean zone of inhibition against the isolates at 100 mg/ml, while the chloroform extract exhibited the lowest zone of inhibition at the same concentration.

3.5 Mean Zones of inhibition of typed *Staphylococcus aureus* (ATCC 25923) to extracts of *Vernonia amygdalina* at 50 mg/ml

The result of the mean zones of inhibition of *Vernonia amygdalina* at 50 mg/ml extracts to typed *Staphylococcus aureus* (ATCC 25923) isolates is presented in Fig. 3. The ethanolic extract of *Vernonia amygdalina* gave the highest mean zone of inhibition against the isolates at 50 mg/ml, while the chloroform extract exhibited the lowest zone of inhibition at the same concentration.

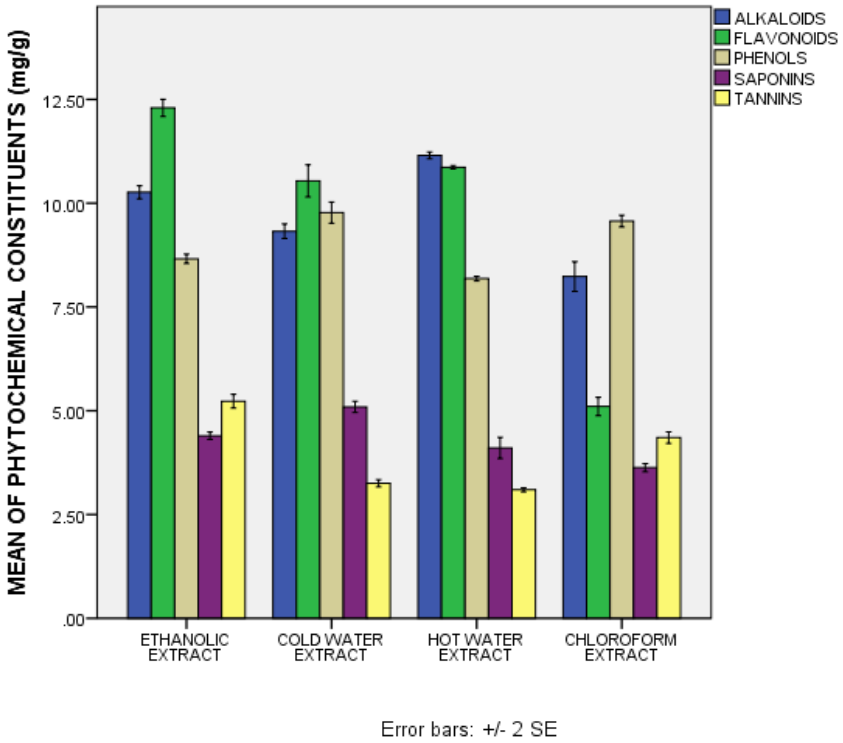

Fig. 1. Quantitative phytochemical composition of *Vernonia amygdalina* extracts

Table 3. Minimum inhibitory concentrations of *Vernonia amygdalina* extracts on *S. aureus* from wounds

S. aureus source	Ethanol (mg/ml)	Cold-water (mg/ml)	Hot-water (mg/ml)	Chloroform (mg/ml)
Accident wounds	12.5 - 50	50 - 100	50 - 100	50 - 100
Post-operative wounds	12.5 - 50	25 - 50	25 - 50	50 - 100
Burn wounds	25 - 50	50	50	100
Vaginal delivery wounds	12.5 - 25	25 - 50	25 - 50	50

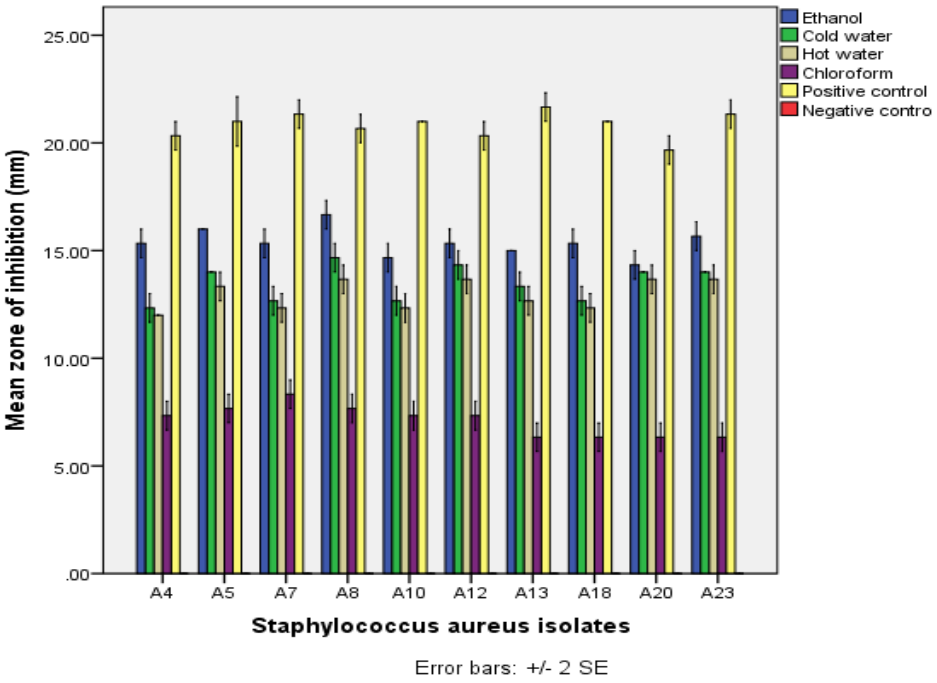

Fig. 2a. Zone of inhibition of *Vernonia amygdalina* extracts at 100 mg/ml on *S. aureus* isolates from accident wounds

Legend: Positive control = Ofloxacin/Vancomycin, Negative control = Dimethylsulphoxide (DMSO), negative Control in all cases yielded no inhibition.

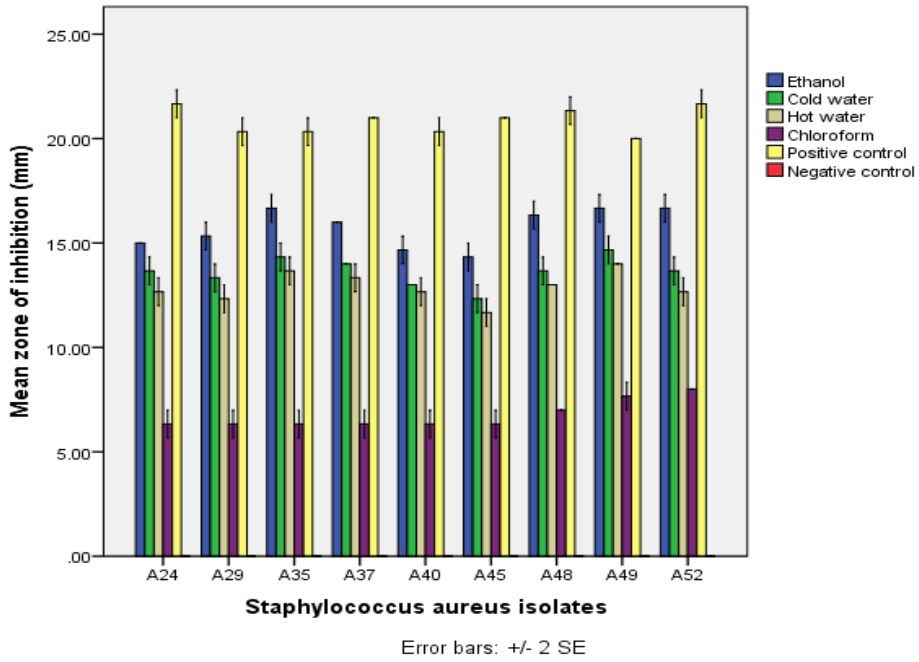

Fig. 2b. Zone of inhibition of *Vernonia amygdalina* extracts at 100 mg/ml on *S. aureus* isolates from accident wounds

Legend: Positive control = Ofloxacin/Vancomycin, Negative control = Dimethylsulphoxide (DMSO), negative control yielded in all cases no inhibition

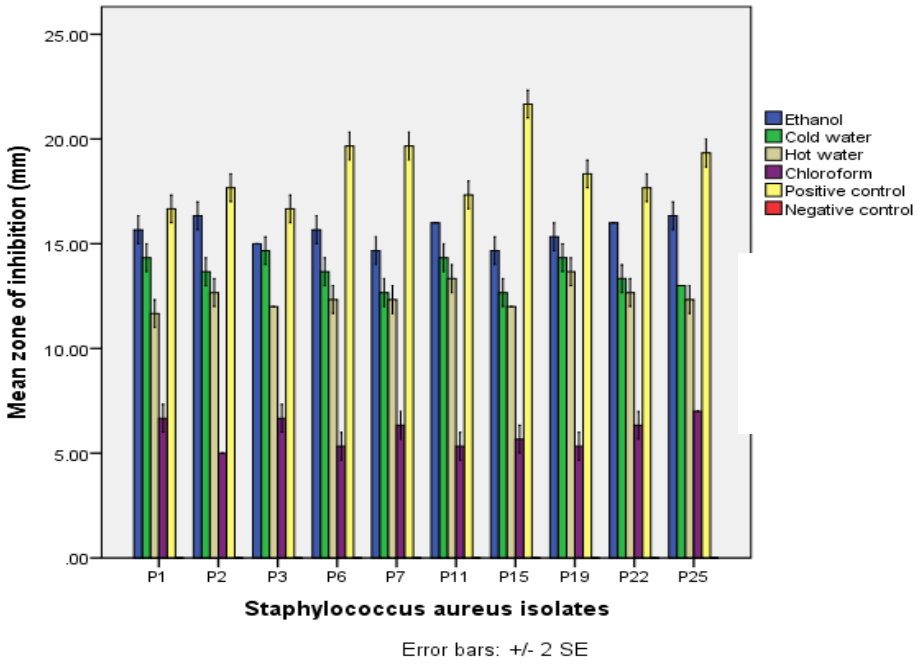

Fig. 2c. Zone of inhibition of *Vernonia amygdalina* extracts on *S. aureus* isolates from post-operative wounds

Legend: Positive control = Ofloxacin/Vancomycin, Negative control = Dimethylsulphoxide (DMSO), negative control yielded in all cases no inhibition

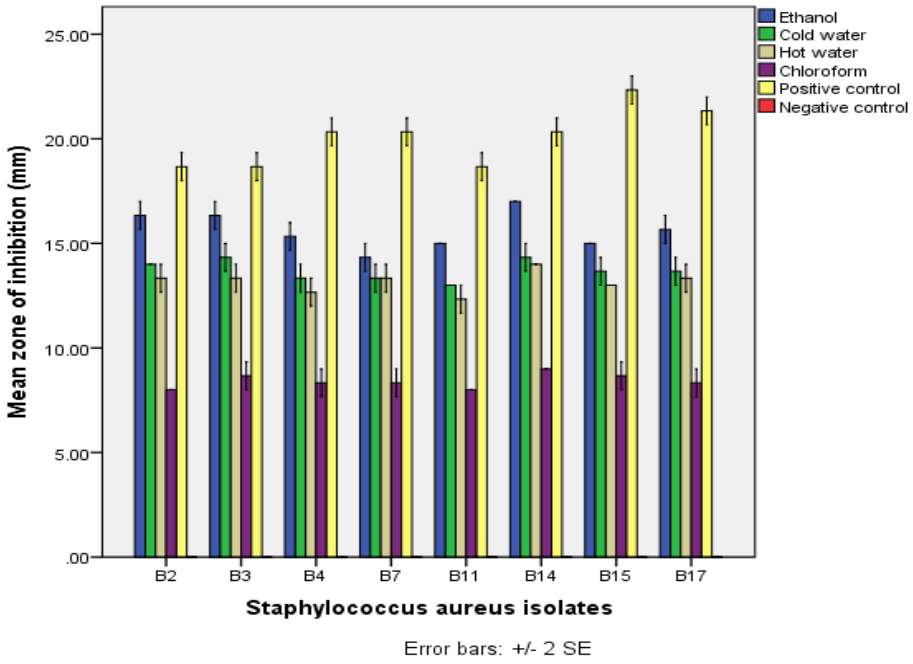

Fig. 2d. Zone of inhibition of *Vernonia amygdalina* extracts at 100 mg/ml on *S. aureus* isolates from burns

Legend: Positive control = Ofloxacin/Vancomycin, Negative control = Dimethylsulphoxide (DMSO), negative control yielded in all cases no inhibition

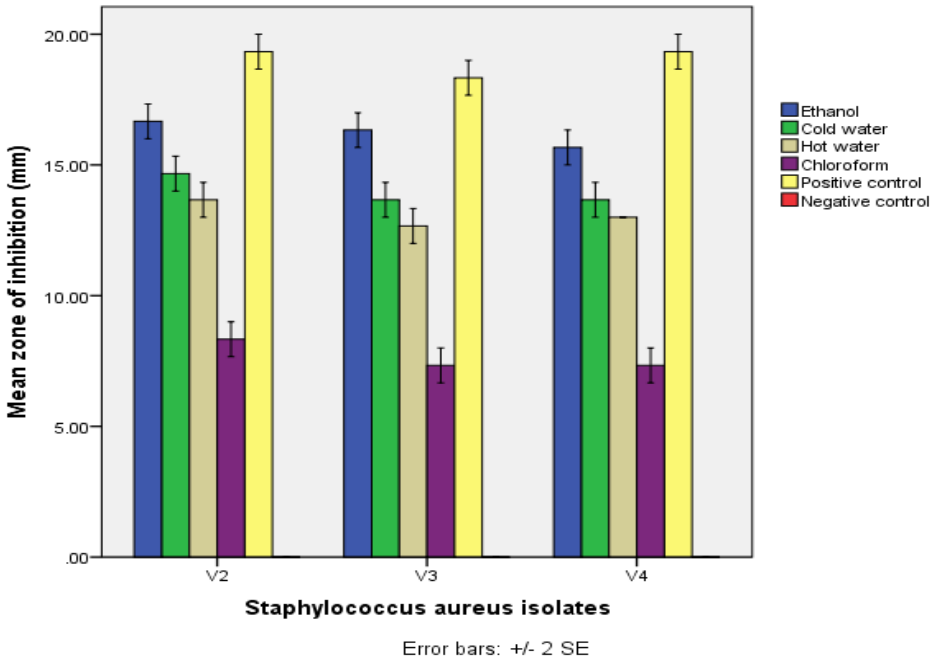

Fig. 2e. Zone of inhibition of *Vernonia amygdalina* extracts at 100 mg/ml on *S. aureus* isolates from vaginal delivery wounds

Legend: Positive control = Ofloxacin/Vancomycin, Negative control = Dimethylsulphoxide (DMSO), negative control yielded in all cases no inhibition

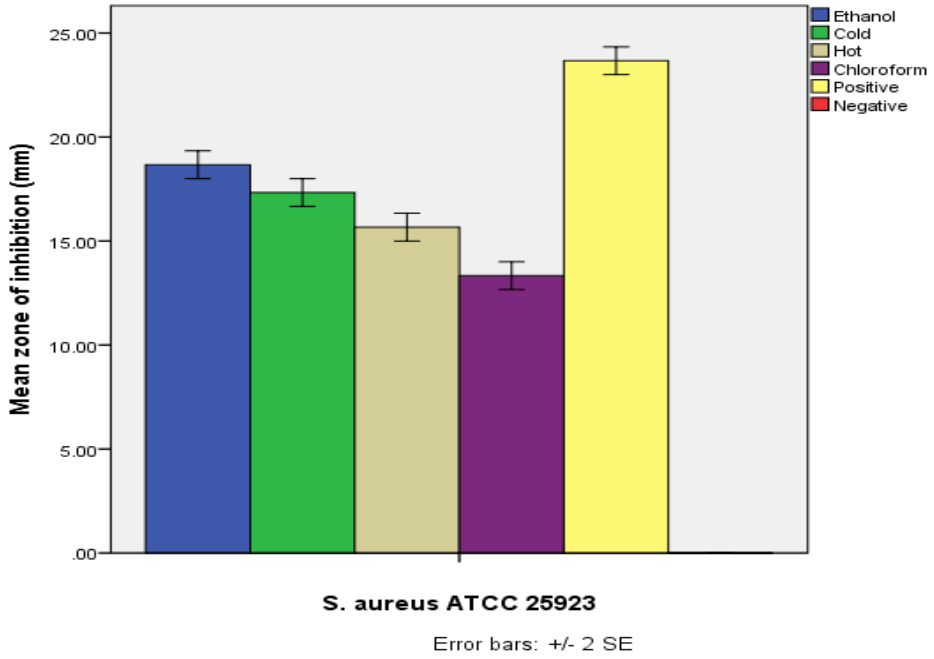

Fig. 3. Zone of inhibition of *Vernonia amygdalina* extracts at 50 mg/ml on *S. aureus* ATCC 25923

Legend: Positive control = Ofloxacin, Negative control = Dimethylsulphoxide (DMSO), negative control yielded in all cases no inhibition

Table 4. Minimum bactericidal concentrations of *Vernonia amygdalina* extracts at 100 mg/ml on *S. aureus* isolates from wounds

S. aureus source	Ethanol (mg/ml)	Cold-water (mg/ml)	Hot-water (mg/ml)	Chloroform (mg/ml)
Accident wounds	50 – 100	100 – 200	100 – 200	100 - 200
Post-operative wounds	25 – 100	100	100	200
Burn wounds	50 – 100	100	100	200
Vaginal delivery wounds	25 - 50	50 - 100	50 - 100	100 - 200

3.6 Minimum Inhibitory and Minimum Bactericidal Concentrations of *Vernonia amygdalina* Extracts on *Staphylococus aureus* Isolates from Wounds and Typed Isolate of *Staphylococus aureus* ATCC 25923

The minimum inhibitory concentration of the extracts vary between 12.5 and 100 mg/ml; while the minimum bactericidal concentration of extracts vary between 50 and 200 mg/ml. The results are presented in Tables 3, 4 and 5.

Table 5. Minimum inhibitory and minimum bactericidal concentrations *Vernonia amygdalina* extracts on typed *S. aureus* ATCC 25923

Extract	MIC	MBC
Ethanol	12.5	25
Cold Water	25	50
Hot Water	25	50
Chloroform	50	100

The results of the susceptibility testing of the isolates to *Vernonia amygdalina* showed that the leaf extracts of the plant possess strong activity against the tested isolates. This corroborates the previous findings of [20] which revealed that the ethanol and aqueous extracts of *Vernonia amygdalina* was active against *Escherichia coli* and *Pseudomonas aeruginosa*. The ethanol leaf extract of *Vernonia amygdalina* at 100 mg/ml exhibited significantly (P<0.05) better zone of inhibition against the tested clinical and typed isolates of *S. aureus* compared to other extracts (cold water, hot water, chloroform). This agrees with the findings of [27] who found that the ethanol extracts of *Vernonia amygdalina* was more active than its water extract in inhibiting the growth of *Vibrio cholerae* and *Escherichia coli.*

The better activity of the standard antibiotics (ofloxacin and vancomycin) over the extracts could be due to the fact that organic extracts are in crude form compared to synthetic antibiotics which have high degree of purity; hence the secondary active metabolites could be present in low concentration or even be masked in the extracts [26].

4. CONCLUSION

The results of this study suggests that *Vernonia amygdalina* leaf extracts can be used as potential herbs for drug development for the treatment of infections caused by *Staphylococcus aureus*. This may also help reduce the overdependence on commercial antibiotics and cases of antibiotics resistance.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this paper.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 declaration of helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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