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Antimicrobial Activity of *Vernonia amygdalina, Justicia betonica, Leonatis nepetaefolium* and *Mormadica foetilda*to*Staphylococcus aureus*

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Abstract
Background: *Many societies in Africa still apply local medicines against numerous infections(1). Traditional medicine/herbs such as Vernonia amygdalina, Justicia betonica, Leonatis nepetaefolium and Mormadica foetilda possess active compounds that combat these life threatening infections.(2) The rural Africans are ignorant about the best mode of extracting these plant remedies. The aim of this study was to assess the antimicrobial activity of traditionally extracted crude herbal plant remedies of Vernonia amygdalina, Justicia betonica, Leonatis nepetaefolium and Mormadica foetilda on staphylococcus aureus.*
Methods*: 180 fresh and dry uninfected leaves were collected by purposive sampling. 45 leaves for each herb were selected and 15 leaves were used for each method of extraction. Each mode of extraction was tested on two culture plates inoculated with S. aureus. The plant leaves were prepared by sun drying the fresh leaves for 5 days and then pounding to obtain a powder which was then mixed with distilled water, secondly squeezing fresh leaves to obtain an infusion and finally crushing fresh leaves and boiling them in a conical flask to obtain a decoction. The obtained mixture, infusion and decoction were tested for antimicrobial activity against S. aureus inoculated culture plates in an incubator at temperature of 37^{o}C for 24 hrs.*
Results: *Freshly squeezed leaves produced an infusion which had the highest average diameter of 59.5mm (40%) and dried leaf mixtures followed with an average diameter of 56mm (38%).The extracts obtained from boiled decoction in this study displayed very weak antimicrobial activity with an average of 32mm (22%).*
Conclusion: *S. aureus is susceptible to freshly prepared leaf infusions since they contain all active compounds with antimicrobial activity. Dried leaf mixtures possess some active compounds; however, some are lost during the process of drying. For boiled leaf decoctions, most active compounds are volatile hence evaporate off on heating and others maybe protein in nature hence denatured by heat showing a very weak antimicrobial activity against S. aureus.*
List of key words*:Staphylococcus aureus, Justicia betonica, Leonatis nepetaefolium, Mormadica foetilda, Vernonia amygdalina*

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I. Introduction

Traditional herbal medicines are naturally occurring plant-derived substances with minimal or no industrial processing that have been used to treat illness within local or regional healing practices.(3)

The World Health Organization estimates that approximately 80% of the world's inhabitants rely on traditional or herbal medicines for their primary health care and plants have long formed the basis of sophisticated traditional medicine systems and purportedly provide excellent leads for new drug developments(4, 5)

About 70 – 80% of the Ugandan population still relies on traditional healers for day-to-day health care. In some rural areas the percentage is around 90% compared to 80% reported world-wide(6).

Many herbal plants have been known to synthesize active secondary metabolites such as phenolic compound found in essential oils with established potent insecticidal and antimicrobial activities against common microbes such as S. aureus, which indeed has formed the basis for their applications in some pharmaceuticals, alternative medicines and natural therapies(7, 8)

II. Material and Methods

Study design: This was a cross sectional study that determined the susceptibility of S. aureus according to mode of extraction of *Justicia betonica*, *Leonatis nepetaefolium*, *Mormadica foetilda*, *Vernonia amygdalina* plant remedies.

Study location: The studied herbal plants were collected from Wakiso District which is located in Uganda's central region
Study Duration: February 2019 to November 2019

Sample size: 180 plant leavesof 4 medicinal herbs were studied and 45 leaves of each herb were selected. 15 leaves were selected for each method of extraction. Each mode of extraction was tested on two culture plates of S. aureus.
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Sample size calculation: Sample size was calculated using Yamane formula (1967:886). A confidence level of 95% and P = 0 .05 was considered.

$$n = \frac{N}{1 + N * (e)^2}$$

n is the sample size
N is the population size
e is level of precision
$n = 225/1+ 225(0.05)^2$
n = 180
Therefore 180 herbal plant leaves were considered for this study.

Procedure methodology

Collected plant tissues were washed with tap water to remove the dust. The plant tissue was then washed with a detergent (liquid soap) and then rinsed with distilled water 3 times consecutively. The plant tissue was put in clean beakers and each labeled corresponding to the plant species
Generally, three crude modes of extraction were tested and these were air drying of fresh leaves, boiling of fresh leaves and squeezing of fresh leaves.

- **Air-Drying:** The cleaned plant tissues were air dried for 5 days and nights in order to reduce their moisture content. The dried tissue was ground using a motor and pestle and the powder dissolved and kept in a test tube as a mixture. For air-drying to be successful, the plant tissues were put in a low humid place with good air supply.
- **Infusion:** The process of extracting chemical compounds or flavors from plant material in a solvent such as water, oil or alcohol by allowing the material to remain suspended in the solvent over time. This was done by squeezing and grinding fresh plant tissues in a mortar and pestle in order to obtain an infusion. The infusion was put in a clean sterilized test tube and the top sealed using Aluminium foil to prevent contamination.
- **Decoction**: This is a method of extraction done by boiling herbal plant samples in any solvent in order to dissolve its phyto chemicals. Decoction involved first mashing the plant material to allow maximum dissolution, and then boiling in water to extract oils, volatile organic compounds and other various chemical substances. A conical flask was used to boil the plant tissue using water as a solvent. The heat source was a spirit lamp with pure ethanol as fuel.

Media preparation

Using an electronic weighing balance and paper foil, weighed 9.5g of Mueller Hinton Agar and suspended it in 250ml of distilled water in a 250ml media bottle and then heated to dissolve the medium completely. The media was then sterilized by autoclaving at 151bs pressure, temp of 121^{o}C for 15 minutes and then left to cool. At temperatures of 45-50^{o}C, mixed the media well and poured it into dry sterilized Petri plates and left then in the bio safety cabinet to cool and solidify.

Inoculation

Prepared S. aureus inoculum that was equivalent to the concentration of 0.5M McFarland standard. Sterile cotton swabs were used to create a uniform lawn of bacterial inoculum on the MHA media.

Then dug 5 wells in each culture plate using sterile glass cork borers where 3 wells represented the 3 extracts from the 3 methods of extraction and the remaining 2 for a positive control and a negative control respectively. Using a Micro pipette, 10µl of each extract were put into each well respectively and labeled corresponding to the method of extraction or control as shown in the table below.

Table 1 Table showing label codes for the wells

Fresh squeezed	Boiled.	Dried
NS- *J. betonica squeezed*	NB- J. betonica boiled	ND- J. betonica dried
MS- *V. amygdalina squeezed*	MB- V. amygdalina boiled	MD- V. amygdalina dried
BS- *M. foetilda squeezed*	BB- M. foetilda boiled	BD- M. foetilda dried
ES- *L. nepetaefolium*	EB- L. nepetaefolium boiled	ED- L. nepetaefolium dried

For each herb, 2 plates were used and the compliment of the above codes used to identify the wells e.g. NS', MS'.

The plates were then cultured for 24 hrs in an incubator at 37^{o}C to allow antimicrobial activity to take place.

Data entry: The collected data was entered in the log book and subsequently into the computer using Microsoft excel

Data analysis: The study was purely quantitative hence numeric data was generated and subjected to descriptive statistics by summarizing the average diameter of zone of inhibition and also the percentage diameter.

III. Result

Average diameter of zone of inhibition of each herbal plant remedy

Table 2 showing zone of inhibition diameter (mm) of freshly squeezed leaves

EXTRACT CODE	PLATE 1	PLATE 2	AVERAGE
NS	6	6	6
MS	20	21	20.5
ES	18	19	18.5
BS	15	14	14.5
TOTAL	59	60	59.5

According to table 2 above, it shows that freshly squeezed J. betonica had an average diameter of zone of inhibition of 6 mm, *V. amygdalina* 20.5 mm, *L. nepetaefolium* 18.5mm, *M. foetilda* 14.5mm

Table 3 showing zone of inhibition diameter (mm) of boiled leaves

EXTRACT CODE	PLATE 1	PLATE 2	AVERAGE
NB	7	6	6.5
MB	10	11	10.5
EB	7	9	8
BB	7	7	7
TOTAL	31	33	32

According to table 3, it shows that boiled plant extracts had average diameters of zone of inhibition as; J. betonica 6.5mm, *V. amygdalina* 10.5mm, *L. nepetaefolium* 8mm, *M. foetilda* of 7mm.

Table 4 showing zone of inhibition diameter (mm) of dried leaves

EXTRACT CODE	PLATE 1	PLATE 2	AVERAGE
ND	15	16	15.5
MD	24	25	24.5
ED	0	3	1.5
BD	15	14	14.5
TOTAL	54	58	56

According to table 4 above, its shows that dried leaves of *J. betonica* had an average diameter of 15.5mm, *V. amygdalina* had 24.5mm, *L. nepetaefolium* had 1.5mm and *M. foetilda* had 14.5mm.

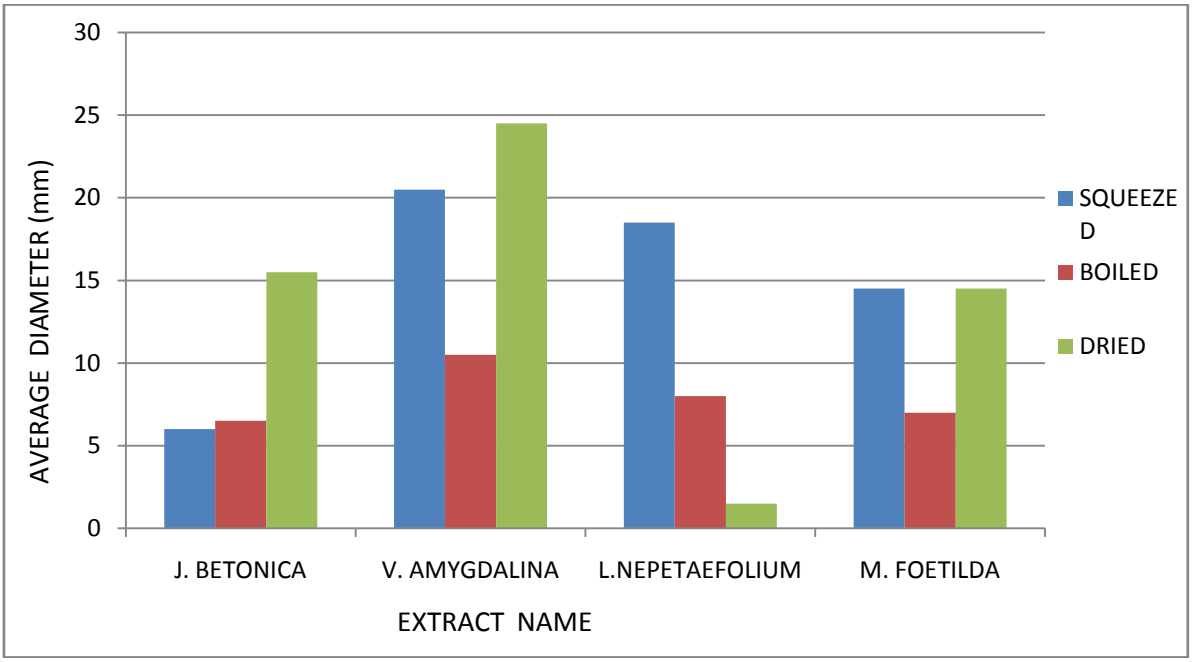

Figure 1: A graph showing average diameter of zone of inhibition of each plant extract

The medicinal plant extracts with the highest antimicrobial activity on *Staphylococcus aureus* culture was *V.amygdalina* with an average diameter of 55.5mm and the least was diameter was recorded in *J.botanica* and *L.nepetaefolium* 28mm.

Table 5 showing total average diameter of zone of inhibition per plant species

Extract name	Total diameter (mm) in plate 1	Total diameter (mm) in plate 2	Total average diameter (mm)
J. betonica	28	28	28
V. amygdalina	54	57	55.5
L. nepetaefolium	25	31	28
M. foetilda	37	35	36

The most effective crude method of extraction of selected herbal plant extracts was squeezing the fresh leaves in which the extract had an average diameter of 40mm and boiling was the least effective with a diameter of 22mm.

Table 6 showing percentage zone of inhibition of each mode of extraction on S. aureus

Mode of extraction	Total diameter in plate 1	Total diameter in plate 2	Total average diameter	Percentage zone of inhibition
Fresh squeezing	59mm	60mm	59.5mm	40
Boiling	31mm	33mm	32mm	22
Sun drying	54mm	58mm	56mm	38

IV. Discussion

Results displayed in Figure 1 indicated that all herbs possess antimicrobial activity against *S. aureus* as early reported**(9).**On average *V. amygdalina* is the most effective herbal plant against *staphylococcus aureus* with a total average diameter of 55.5mm as noted by zone of inhibition this was in agreement with the findings by Francois et al**(10)**followed by *M. foetilda* with a total average of 36mm and the least effective with a total average of 28mm each were *L. nepetaefolium* and *J. betonica*. This shows that *V. amygdalina* have more active compounds against *S. aureus* while both *M. foetilda* and *L. nepetaefolium* are weak herbs against *S. aureus*.

Results further indicated that all the modes of extraction were effective**(11)**. This was previously reported that plant extracts displayed stronger antimicrobial effect on the gram positive strains than on their gram-negative counterparts**(9, 11)**

Results further showed that on average, freshly squeezed leaves produced an infusion which had the highest average diameter of 59.5mm and dried leaf mixtures followed with an average diameter of 56mm.The extracts obtained from boiled decoction in this study displayed very weak antimicrobial activity with an average of 32mm. These results were in agreement with those of H.V Girish as noted in**(12)**

V. Conclusion

The conducted study found out that herbal medicine is effective towards the treatment of common infections caused by *S. aureus* and that the most effective crude mode of herbal plant extraction is fresh squeezing. Leaf infusions had the highest antimicrobial activity on *S. aureus* hence sooner than expected, broad-spectrum drug which might be able to cure various human ailments could be developed from freshly squeezed *V. amygdalina*, *M. foetilda*, *L. nepetaefolium* and *J. betonica*.

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