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THE EFFECT OF A LOCAL MINERAL *KADOSERO* TOWARDS THE ANTIMICROBIAL ACTIVITY OF MEDICINAL PLANT'S EXTRACT: CASE OF LAKE VICTORIA BASIN, TARIME TANZANIA.

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## Abstract

The effect of *Kadosero*, a crude mineral used by traditional healers as a supplement to plant extracts against microbial infections was evaluated. A sample of *kadosero* from a local market was both analyzed for its basic composition and its role on bioactivity of plant extract. Titrimetric, Gravimetric and Atomic Absorption Spectrometric analyses were used to determine contents of the mineral *kadosero*. Disc Diffusion Assay was used for bioactivity screening *in-vitro*. Chemical analysis of *kadosero* revealed the presence of  $SO_4^{-2}$  (0.0038mg/g),  $Fe^2$  (0.0027mg/g), Cl<sup>-</sup> (232.683mg/g) and Na<sup>+</sup> (151.25mg/g). *In-vitro* tests revealed that supplementing extract of *Balanites aegyptiaca* with a mineral *kadosero* by using untreated well water reduced number of bacterial from 100 colony forming units to nil at a mass of a mineral between 60-100 mg. On the other hand, a mineral *kadosero* did not increase bioactivity of the extract of *B. aegyptiaca* against the test microbes in agar disc diffusion assay. This was attributed by interaction between the mineral *kadosero* and nutrient agar medium. The crude mineral *kadosero* can be supplemented to other plant extracts used locally for treatment of general bacterial infections for increased bioactivity. Further study is recommended to determine mechanisms for bacterial vulnerability to this mineral supplement.

**Key words:** Mineral supplement; Plant extract; Traditional healers

## Introduction

Traditional healing practices in Lake Victoria basin, Tarime District, Tanzania like elsewhere in developing world encompasses complex practices whereas plants extracts are supplemented with minerals or animal products such as bones, fats and honey depending on the ailment (Otieno *et al.*, 2001). The common mineral supplement used in such encounter within Lake Victoria basin is locally known as *Kadosero* in Luo. The mineral *kadosero* is excavated from the specific sites in the region, packed and sold at local markets at \$ 2.5 for a 300g bolus. Medicinal plant extracts commonly supplemented with this mineral *kadosero* according to this study are powdered stem bark of *Balanites aegyptiaca* and powdered seeds of *Abrus precatorius* against respiratory infection. Powdered leaves of *Commiphora africana* and *Mangifera indica* are also mixed with a mineral *kadosero* treat burns, sores or wounds. In a similar application in Siaya district-Kenya, the powdered bark of *Balanites aegyptiaca* is mixed with a mineral *Kadosero* and then licked as a remedy against respiratory infections (Johns, *et al.*, 1990).

According to the website NAPRALERT, *B. aegyptiaca* itself is a potential antimicrobial agent. However, there is scanty scientific report on the role of local minerals on plant extracts against pathogenic microbes. This study sought to evaluate scientifically the contribution of the mineral *kadosero* to the bioactivity of plants with antimicrobial activity. The experiment compared bioactivity of plant extracts supplemented with a mineral *kadosero* and the one without a mineral. As long as local communities in Tanzania like other developing countries keep on relying on medicinal plant extracts for curative of common ailments, then availability of medicinal plant supplements ensures increased biological activity of plant extracts in traditional healing systems.

## Material and methods

The ethnomedicinal information on the use of a mineral *kadosero* was obtained from local communities in Tarime District in Lake Victoria basin, Tanzania. The research was carried out from March 2004 to September 2005. The voucher specimen of *Balanites aegyptiaca* was authenticated at University of Dar es Salaam herbarium where the voucher specimen was then deposited. The mineral *kadosero* was purchased at a local market in Ochuna village in Tarime District, Tanzania. A sample of the mineral *kadosero* was analyzed at the Department of Chemistry, University of Dar es Salaam to identify its ingredients. The techniques according to Emteryd (1989) and Vogel (1961) were used to analyze components of the crude mineral *kadosero* as follows: Simple gravimetric analysis was used to determine moisture content, atomic absorption spectroscopy was used to determine proportions of iron and sodium and titrimetric analysis was used to determine presence and amount of chloride ions.

Bark samples of *Balanites aegyptiaca* (L.) Delile (*Balanitaceae*) from Lake Victoria basin were shade dried at room temperature for two weeks before grinding to a size of 60 mesh powder. The powder 400g was then extracted with 2,500ml of 95% ethanol using a soxhlet apparatus for 48hrs according to Draper (1976) at the Department of Chemistry, University of Dar es Salaam. The products were evaporated *in vacuo* to a brown oily extract 4.32g using a rotary evaporator at 40°C. In order to assess the contribution of a mineral supplement on bioactivity of plant extract, the test was carried out in two phases. Phase one involved direct application of mineral and/or plant extract on untreated water to avoid the possible inhibition of nutrient agar that could constrain activity of the mineral. Phase two according to Platt (1986) involved agar disc diffusion assay where the plant extracts with and without mineral *kadosero* were tested against the standard laboratory microbes.

### 2.1. Direct application of plant extract plus a mineral (*Kadosero*) on untreated well water without agar medium

Water samples 200ml were collected from each of the six shallow wells in different locations in Dar es Salaam city. Initially, 0.2ml from each of these water samples was overlaid on sterile nutrient agar plates, and then incubated for 24 hours at  $37 \pm 1^{\circ}\text{C}$ . The purpose was to assess the presence of microbes in these untreated water samples. After incubation for 24hrs, number of bacterial Colony Forming Units (C.F.U) for six samples was counted. The water sample with highest number of C.F.U was selected for further screening. 50ml out of this selected water sample was treated with plant extract or mineral *kadosero* as follows;

- Plant extracts 5, 50, 500 and 1000mg without mineral supplement.
- Mineral *kadosero* 15, 30, 60 and 100mg without plant extract.
- Constant plant extract of 60mg supplemented with 15, 30, 60 and 100g of mineral *kadosero*.

Triple treatments were incubated for 24hrs at  $37 \pm 1^{\circ}\text{C}$  after which, the sample from each treatment was now then overlaid on sterile nutrient agar and incubated for 24hrs to assess the presence of bacteria through colonies count. Each treatment was repeated twice, after which the average readings for bacterial counts and their standard deviations determined as presented in Table 2.

### Agar diffusion test

In agar disc diffusion assay, each test microbe was subjected to three treatments as follows;

- Plant extract (10mg/ml) without mineral *kadosero*
- Plant extract (10mg/ml) with additional 30, 60 and 100mg mineral *kadosero*.
- Mineral *kadosero* alone at the concentrations of 30, 60 and 100mg/ml in distilled water

The test microbes in agar disc diffusion assay were gram negative bacteria namely *Escherichia coli* (DSM 1103), *Pseudomonas aeruginosa* (DSM 1117), *Salmonella typhi* (NCTC 8385) and gram positive bacteria; *Staphylococcus aureus* (ATCC 25923), *Klebsiella pneumoniae* (NCTC 9633) and local strain of *Streptococcus faecalis*.

The plant extracts and mineral *kadosero* were impregnated in standard filter paper disc (7mm diameter). For the meantime, nutrient agar media plates were flooded with 0.1 - 0.2 ml of the test inoculums ( $10^5$  C.F.U) in nutrient broth after which the activity of antimicrobial agents were indicated by clear zones of inhibition and were measured in millimeter by a transparent ruler after 24 hours incubation at  $37 \pm 1^\circ\text{C}$ . The analysis of variance was used to compare bioactivity results of treatment means on selected microorganisms. The null hypothesis was that the variance of the bioactivity of plant extract without the mineral *kadosero* equals the extract without the mineral at a probability level of 5%.

## Results

### Contents of the local mineral *kadosero*

Table 1 presents the contents of the local mineral *kadosero* as was analyzed at Chemistry Department, University of Dar es salaam, Tanzania.

**Table 1:** Contents of a mineral *Kadosero*

Parameter	Conductivity $\mu\text{S}/\text{cm}$	Moisture content% ww	$\text{SO}_4^{-2}$ mg/g	$\text{Fe}^2$ mg/g	$\text{Cl}^{-1}$ mg/g	$\text{Na}^+$ mg/g
amount	12.51	12.696	0.0038	0.0027	232.687	151.25

### Effect of mineral *kadosero* on the bioactivity of plant extract by using untreated water samples (without nutrient agar media)

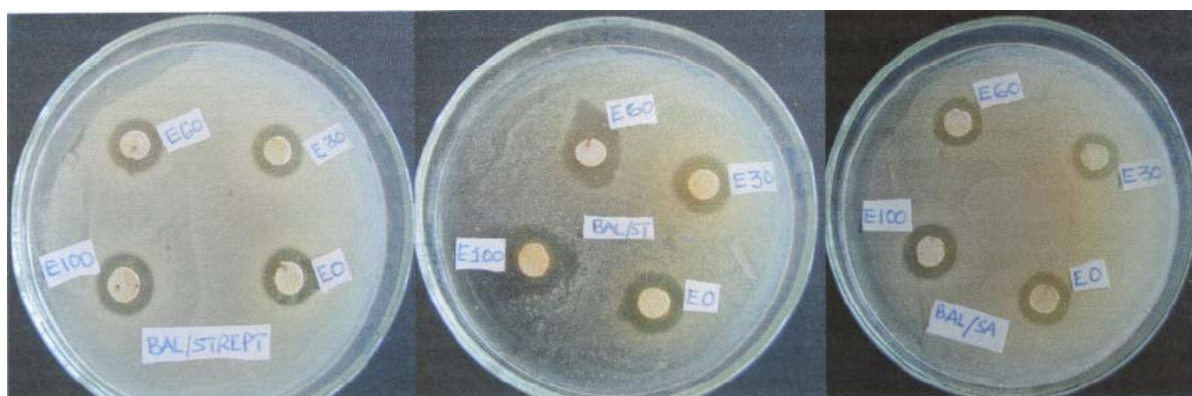
In bioassay test by using untreated water samples, bacteria colonies declined with increased concentration of plant extract. However, the decline in bacterial colonies was accelerated with additional mineral *kadosero*. For example, when 60mg of plant extract was added to 50ml of untreated water, the colony forming units for bacteria remained above 40, however, the count dropped to  $25 \pm 2.8$  C.F.U when the same 60mg of plant extract was supplemented with 60mg of a mineral *kadosero*. The mineral *kadosero* itself showed higher activity than plant extract alone. The activity of plant extract with mineral *kadosero* and the extract without a mineral as applied directly on untreated water samples (50ml each) is summarized in Table 2.

**Table 2:** Effect of a mineral *kadosero* on bacterial growth by using untreated water (without agar medium)

Mineral <i>kadosero</i> without plant extract		Plant extract without a mineral <i>kadosero</i>		Constant plant extract (60mg.l <sup>1</sup> ) plus variable mineral weights	
Mineral (mg)	Bacteria colonies	Plant extract (mg)	Bacteria Colonies	Mineral (mg)	Bacteria colonies
0	uncountable	0	uncountable	0	Uncountable
15	100 $\pm$ 18.4	5	89 $\pm$ 21.2	15	64 $\pm$ 9
30	100 $\pm$ 12.7	60	54 $\pm$ 9.9	30	31 $\pm$ 12.7
60	34 $\pm$ 9.9	500	49 $\pm$ 11.3	60	25 $\pm$ 2.8
100	6 $\pm$ 7	1000	26 $\pm$ 5.7	100	0

### Effect of a mineral *kadosero* to the bioactivity of plant extract by using agar disc diffusion assay.

The extract of *B. aegyptiaca* was active to all test microbes except *E. coli* even without the mineral *kadosero*. Similar observation on insensitivity of *E. coli* to *B. aegyptiaca* extract was reported in Kenya by Taniguchi, *et al.* (1978) and in Sudan by Liu and Nakashi, 1982). The mineral *kadosero* did not increase bioactivity of plant extract in disc diffusion assay against test bacteria (see plate 1). Also, the mineral supplement alone without plant extract at 30, 60 and 100g/ml did not inhibit growth of bacteria indicating that the mineral did not have the inhibition activity against the microbes in agar disc diffusion method. The analysis of variance to compare bioactivity of plain and supplemented extract in agar disc diffusion showed that the computed F value of 0.78 was less than the critical F value of 3.86, ( $P > 0.05$ ), so the null hypothesis was accepted that a mineral *kadosero* did not increase the activity of plant extract by using disc diffusion method. In a similar experiment in Brazil, Estrela *et al.*, (2003) noted that the activity of 2% Sodium Hypochlorite and 2% Chlorhexidine against the test microbes was minimal when agar medium was used as compared to direct exposure. Plate 1 in this study is bioactivity results of plant extract 10mg/ml supplemented with 30, 60 and 100mg of mineral *kadosero*. The labels E0, E30, E60 and E100 in plate 1 stand for plant extract plus 0, 30, 60 and 100gm mineral *kadosero* respectively. The zones of inhibition for different microbes showed that there was no difference in the effect of a mineral *kadosero* to the bioactivity of plant extract on microbes by using agar disc assay.



**Plate 1:** Bioactivity of *B. aegyptiaca* extract at different mineral *kadosero* concentrations i.e. 0, 30, 60 and 100mg/ml on *S. faecalis* (left), *S. typhi* (centre) and *S. aureus* (right).

### Discussion

Basing on the results, the extract of *B. aegyptiaca* was active to the test microbes even before the addition of the mineral supplement. This was attributed by the presence of compounds like sapogenin, triterpene and alkaloids some of which were reported to kill snails, poison fish, kill worms, and to have antivenin activities (Ognyanov *et al.*, 1977; Seida, *et al.* 1981). The laboratory tests have now confirmed that the mineral *kadosero* boosts bioactivity of plant extract as compared to plant extract without a mineral supplement. Basing on the results on the bioactivity tests by using direct application of plant extracts on well water, the mineral *kadosero* can be used to supplement other plant extracts with antimicrobial activity for increased bioactivity in traditional healing systems. Apart from mineral *kadosero* screened in this study, Adefeye and Opiah (2003) found that the common plant supplements including onion, garlic onion, palm oil, ginger, palm kernel oil, lime, bitter kola, sugar and honey were very popular in the treatment of the common cold and cough. Honey like mineral *kadosero* has been used as a supplement against microbial upper respiratory infections and wounds for quite long. The antibacterial activity of

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honey is attributed to a number of reasons including high acid content, high osmotic pressure and the "inhibine effect". The latter is due to the hydrogen peroxide produced and accumulated in honey (While and Doner, 1980).

Despite that the mineral *kadosero* had the inhibitory effect to the growth of microbes through direct exposure to the water samples, the failure of the mineral *kadosero* to increase bioactivity of the extract of *B. aegyptiaca* in agar disc assay was the indication that the agar media inhibited expression of the mineral against the test microbes. The idea is substantiated further by findings of Estrela *et al.*, (2003) in a different study that solubility and diffusibility of minerals was low in agar medium. It is most likely that the agar medium only constrained diffusion of the mineral *kadosero* than deactivating it. The above direct exposure proved that the mineral had inhibitory activity to the growth of microbes. Likewise, other minerals with similar constituents to *kadosero* i.e. Sulfur dioxide, sulfites and sodium chloride are used to preserve food due to their inhibitory activity to microbes. Their targets for inhibition include the cytoplasmic membrane, DNA replication, protein synthesis and various enzymes in the cytoplasm of microbes (University of Wisconsin-Madison, 2005).

From this study, it has been learnt that some traditional healing practices may be declared as inactive if verified by using modern and sophisticated laboratory techniques, some of which may end up constraining expression of their activity. The experiments meant to verify traditional healing systems should start by mimicking closely the indigenous practices before advancing further to more standard protocols. Nevertheless, further study is recommended to determine chemical and physiological mechanisms by which microbes are becoming more vulnerable to plant extracts that are supplemented with a crude mineral *kadosero* than the plant extract without a mineral supplement.

## Conclusion

The mineral supplement *kadosero* significantly increases activity of the plant extract *Balanites aegyptiaca* against the standard laboratory microbes. This increased bioactivity substantiates the traditional art of supplementing the plant extracts with the mineral. It can now be concluded that the use of a mineral *kadosero* by traditional healers contribute to the healing effect when a powdered bark of *B. aegyptiaca* is used to cure upper respiratory infections.

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