Evaluation of the antibacterial potential of *Vernonia Amygdalina* on foodborne pathogens isolated from kunu sold in Calabar, Nigeria

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ABSTRACT

The antimicrobial efficacy of the formaldehyde, petroleum ether and hexane extracts of *Vernonia amygdalina* on bacteria isolated from a popular local beverage “kunu” sold in various locations in Calabar Metropolis was evaluated. The antibacterial activity was compared with standard antibiotics. Bacteriological assessment of kunu was conducted using standard sensitivity test methods. The results revealed the presence of various species of bacteria including *Bacillus cereus*, *Escherichia coli*, *Lactobacillus* sp., *Staphylococcus aureus*, *Streptococcus fecalis*, *Bacteriodes fragilis*, *Clostridium perfringens*, *Bacillus subtilis*, *Corynebacterium* sp. and *Micrococcus* spp. The antibacterial activity of the formaldehyde extract of *V. amygdalina* inhibited some of these bacteria to a greater extent than the other extracts and standard antibiotics. Plants are rich source of antibacterial agents, which could be exploited in human disease management and many edible vegetables in Nigeria have been known to have antibacterial potentials. The presence of bacteria and the level of antibacterial activity of *V. amygdalina* on them signify the health benefit of this particular vegetable and thus the public health importance of the preparation and consumption of kunu.

Keywords: *Vernonia amygdalina*, *Staphylococcus*, kunu, public health, antibiotics

INTRODUCTION

Kunu is a common refreshing health drink hawked in many Nigerian cities and towns. It is made with cereals like millet, sorghum and maize and thus can be used as a food drink. Cereals and cereal products which includes grains, flours, bread and other bakery products contain organisms from soil, insects and other sources. Bacteria found on freshly harvested grains include species of *Pseudomonas*, *Micrococcus*, *Lactobacillus*, *Bacillus* etc. and the total number of bacteria per gram of grain could vary from a few to several millions. Kunu is however produced locally by anyone with the knowledge involved and bottled in reusable empty plastic bottles originally used for bottled water. The poor shelf life and crude packaging method employed in its production leaves a lot to be desired microbiologically. “Kunu” which used to be consumed mainly in northern Nigeria is now widely accepted everywhere in Nigeria (1, 2) and yet there is still inadequate information the microbial load of kunu and the health implications that kunu poses. Effieuwere and Akona, (1995) The microbiological assessment of “kunun zaki” sold in Jos in northern Nigeria during its production process and its health implication has been done previously (3).

*V. amygdalina* is known as mujonso in Tanzania while in Nigeria the Edos call it oriwö, the Hausas call it chusar doki and the Ibibios atidot. In Igbo language it is onubu while the Yoruba call it ewuro.

*Vernonia amygdalina* is a shrub that can reach twenty three feet in height when fully grown and grows throughout tropical Africa and has been domesticated in many parts of West Africa and Nigeria, where it is locally known as
bitter leaf and used as vegetable in soups (4). It has a grey or brown coloured bark. The bark has a rough texture and is flaked. The branches of the shrub are brittle and break off easily. The green leaves are oblong to lanceolate in shape, veined and bear pale soft hairs on the underside. *V. amygdalina* has small white flowers which bloom in clusters. It is most often grown as a leaf vegetable. The therapeutic relevance of all parts of bitter leaf as a useful folkmedicine and a popular vegetable adjunct in the delicious onubu soup has been reported by some researchers (5, 6, 7, 8).

Many plants used in traditional medicine in Nigeria are readily available in rural areas as every day edible vegetables and at relatively cheaper cost than orthodox medicine. Due to a rise in the rate of food borne infections, antibiotic resistance in bacteria and side effects of synthetic antibiotics, medicinal plants are gaining popularity over standard antibiotics.

In this study, formaldehyde, hexane and petroleum ether extracts of *V. amygdalina* were screened for antibacterial activity against bacterial isolates from the popular refreshing fermented drink called “kunu”.

**MATERIALS AND METHODS**

**Sample collection**

Kunu was bought from commercial vendors from four different locations in Calabar including Bogobiri, Etim Edem Park, Watt Market and Ekpo Abasi Junction and taken to the laboratory for direct examination. *V. amygdalina* was harvested from the botanical garden of Cross River University of Technology, Calabar, Nigeria.

**Bacteriological examination of kunu samples**

Bacterial assessment was done for each of the sample using standard bacteriological procedures including serial dilutions, Grams reaction, sugar fermentation and IMVIC test to identify bacterial isolates.

**Extract preparation**

Fresh and air-dried leaves of *V. amygdalina* were used. Leaves were air-dried for several days away from direct sunlight until brittle enough to break. Formaldehyde, petroleum ether and hexane were used for extracting freshly harvested macerated and previously air-dried grounded leaves of *V. amygdalina*. The crude extracts of the leaves were prepared using standard procedures with slight variations (9) involving soaking powdered leaves (20g) in 100ml of solvents for 24h at room temperature. The mixture was filtered and the filtrate evaporation using a rotary evaporator (STUARC SCIENTIFIC, ENGLAND). The residue was retained as the crude extract.

**Test for antibacterial activity of *V. amygdalina***.

The disc diffusion technique using the Kirby-Bauer method as described in Prescott *et al.*, (2005) (10) was used in testing the kunu isolates for their antimicrobial sensitivities. Sensitivity discs were punched from Whatman No. 1 filter paper. The discs were sterilized and afterwards impregnated with the crude extracts of *V. amygdalina* (11). The kunu bacterial isolates were re-suspended in Mueller Hinton broth and incubated for 4h. About 0.3mls of the suspension was dispensed into sterile Petri dishes and molten Mueller Hinton agar No.2 pour over the plate and stirred briskly to make an even distribution of bacteria in culture. The extract impregnated disks were then placed on the surface of each plate and incubated at 37 °C for 24 h. Standard antibiotics sensitivity discs were used as controls for comparison with the extracts. The plates were observed for zones of inhibition after incubation followed by measurements and calculation of the mean zones. Minimum inhibitory and bactericidal concentration values were also determined by broth dilution assay.

**RESULTS AND DISCUSSION**

The probable and identified bacterial isolates and their percentage occurrence in the samples are given in Tables 1 and 2. Bacteria identified include *Bacillus* spp, *Escherichia coli*, *Lactobacillus* spp., *Staphylococcus aureus*, *Streptococcus* spp., *Bacteriodes* spp., *Clostridium perfringens*, *Bacillus subtilis*, *Corynebacterium* spp. and *Micrococcus* spp. *Staphylococcus aureus* had the highest occurrence (30%) among the isolates from the kunu samples followed by *Bacillus* spp. (20%). *Micrococcus* spp. and *Lactobacillus* spp. were not significantly present in the samples and are therefore not reckoned with in this evaluation even though the presence of *Lactobacillus* spp. maybe responsible for the slightly acidic nature of kunu. In a recent study of the microbial succession of foofoo, another staple food in Nigeria (12) 8 microbial strains were isolated including the following, *Bacillus subtilis*, *Lactobacillus plantarum*, *Corynebacterium manihoti*, *Leuconostoc mesenteroides*, *Enterobacter aerogenes*, *Aspergillus niger*, *Geotrichum sp.* and *Saccharomyces cerevisiae*.
Table 1: Bacteriological examination of kunu samples and identified isolates

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>GRAM DESCRIPTION</th>
<th>CATALASE</th>
<th>OXIDASE</th>
<th>INDOLE</th>
<th>CITRATE</th>
<th>MR</th>
<th>PROBABLE ORGANISM</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA* I</td>
<td>G- ve rods occurring in chains</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>Bacteriodes spp</td>
</tr>
<tr>
<td>NA II</td>
<td>Short rods, Long slender rods without spores</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lactobacillus Spp, Corynebacterium spp</td>
</tr>
<tr>
<td>NA III</td>
<td>Clustered appearance, Curved big head with tiny drum stick appearance with spores</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Staphylococcus aureus, Clostridium perfringes</td>
</tr>
<tr>
<td>NA IV</td>
<td>Gram positive, Black colonies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Streptococcus spp</td>
</tr>
<tr>
<td>NA V</td>
<td>G+ve bacillus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Clostridium perfringes, Bacillus subtilis</td>
</tr>
<tr>
<td>NA VI</td>
<td>In clusters, Red coloured cells</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Gram-ve short rods, E. coli</td>
</tr>
<tr>
<td>NA VII</td>
<td>Long slender rods</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td>Lactobacillus spp, Corynebacterium spp</td>
</tr>
</tbody>
</table>

*=Nutrient agar

Table 2: Bacterial isolates and frequency of occurrence

<table>
<thead>
<tr>
<th>Bacteria isolates</th>
<th>% occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>10</td>
</tr>
<tr>
<td><em>Bacteriodes</em> spp.</td>
<td>10</td>
</tr>
<tr>
<td><em>Corynebacterium</em> spp.</td>
<td>15</td>
</tr>
<tr>
<td><em>Streptococcus</em> spp.</td>
<td>10</td>
</tr>
<tr>
<td><em>Bacillus</em> spp.</td>
<td>20</td>
</tr>
<tr>
<td><em>Clostridium perfringes</em></td>
<td>10</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>30</td>
</tr>
</tbody>
</table>

The zones of inhibition obtained for the crude extract of *V. amygdalina* on the test bacterial isolates are shown in Table 3. The highest activity was observed in the formaldehyde extract against *E. coli*. Petroleum ether extracts did not have any activity against the isolates while hexane extract activity was comparatively lower against isolates than formaldehyde extracts. This outcome shows that leaf extracts of *V. amygdalina* possess inhibitory potentials against kunu bacterial isolates.

In Table 4, the minimum inhibitory and bactericidal concentrations are given. The plant shows good inhibitory activity against the isolates tested.

The antibiogram profile of the isolates shows that the leaves of *V. amygdalina* have greater antibacterial activity than most conventional antibiotics tested as presented in Table 5. This agrees with a previous observation (13) that some plant extracts are more potent than some antibiotics.

Table 3: Antibacterial activity of *V. amygdalina* on kunu isolates

<table>
<thead>
<tr>
<th>Isolates/Solvent extracts</th>
<th>Fresh sample</th>
<th>Air-dried sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FE'</td>
<td>HE Zones of Inhibition (mm)</td>
</tr>
<tr>
<td>E. coli</td>
<td>31 20</td>
<td>40 20 -</td>
</tr>
<tr>
<td><em>Clostridium</em> spp.</td>
<td>40 -</td>
<td>40 - -</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>- -</td>
<td>30 20 -</td>
</tr>
<tr>
<td><em>Bacillus</em> subtilis</td>
<td>40 -</td>
<td>33 25 -</td>
</tr>
<tr>
<td><em>Staphylococcus Aureus</em></td>
<td>40 -</td>
<td>30 - -</td>
</tr>
</tbody>
</table>

*FE' – Formaldehyde extract, HE – Hexane extract, PE – Petroleum ether extract*
It has also been recorded (14) that acetone and alcohol extracts of *V. amygdalina* showed higher antifungal activity against *S. cerevisiae* than ciprofloxacin and gentamycin. Acetone-ethanol extract of the leaf and stem of *V. amygdalina* has also previously been shown to have a higher zone of inhibition for *Klebsiella sp* isolated from urine at 20 mg/ml than ciprofloxacin at the same concentrations (15).

Antimicrobial activities in plants have been reported to be as a result of bioactive components present in the plants, such as alkaloids, saponins, tannins, antrachquinones, steriods, flavonoids (16, 17, 18). Leaf extracts of *V. amygdalina* in this study has been shown to inhibit bacteria except for petroleum ether extracts, indicating its broad spectrum activity as an antibiotic.

This result corresponds to earlier works (2, 3) which isolated similar bacteria from kunu sold in Jos area of Nigeria. These bacteria can therefore be assumed to be common food pathogens. *S. aureus* and *E. coli* are common food poisoning bacteria (19) and their presence in kunu signifies a serious health implication in terms of the level of hygiene maintained during its preparation and the potential for a large scale bacterial epidemics. However, lactic acid bacteria of the genus *Lactobacillus sanfransiscensis*, *L. farcininis*, *L. fermentum*, *L. brevis*, *L. plantarum*, *L. amylovorus*, *L. reuteri*, *L. pontis*, *L. panis*, *L. Alimentarius* and *Weissella cibaria* has been shown to be involved in fermentation of cereals (20).

Earlier assessment of the antibacterial activity of *V. amygdalina* leaves extract on selected food pathogens (21) revealed relatively high zones of inhibition at low concentrations, proving that the plants can be medically useful.

**CONCLUSION**

Until now, kunu production and marketing has remained a cottage affair. The benefit and need for a cool and refreshing drink of kunu during the heat of the day cannot be overemphasised. However, considering the rich nutritional content of the popular food drink, kunu serves as a fodder for bacterial growth and proliferation, a fact that needs serious and urgent quality control attention and the need for government regulatory agencies to wade into the cottage industry with possible potentials for better production and packaging process for kunu, thus ensuring a healthier and more nutritious food drink made from Nigerian grown cereals.

**REFERENCES**


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