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Susceptibility of *Citrobacter koseri, Salmonella typhi* and *Klebsiella pneumonia* to Crude Extracts of *Beta vulgaris* (Linn) (Beetroot)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JOI and PAB designed the study, performed the statistical analysis, wrote the protocol and first draft of the manuscript. Authors JOI and PAB managed the analyses of the study. Author LYA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study is to ascertain the antimicrobial action of crude extracts of *Beta vulgaris* (Beetroot) against *Salmonella typhi, Klebsiella pneumonia,* and *Citrobacter koseri.*
Place and Duration of the Study: This study was carried out in the Department of Biological Sciences, Bingham University, Karu, between July to August, 2017.
Materials and Methods: Beetroot samples were obtained from GaddaBiu market Jos. The isolates were obtained from the Zanklin research Institute, Bingham University. Crude extracts of Beetroot bulb was obtained by soaking, boiling and maceration, which was used to determine the susceptibility of the microbial isolates to the extracts.
Results: The highest mean zone of inhibition obtained was 6.67mm from application of boiled crude extracts of *Beta vulgaris* to *Citrobacter koseri*. The highest mean zone of inhibition of antibiotics was 43.67 mm observed from ciprofloxacin upon its application on *Salmonella typhi.* The lowest mean zone of inhibition was 23.00 mm obtained from gentamicin upon its introduction on *C. koseri.*

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Conclusion: The relative antibacterial activity of Beetroot (*Beta vulgaris*) against bacteria has indicated a wide potential as an effective plant which can serve as an alternative to resistant antibiotics.

Keywords: Antibacterial; antimicrobial; soaked; beetroot.

1. INTRODUCTION

The traditional use of plants as a source of inspiration for novel drug compounds has been and is still continually explored. As plant derivatives are used for medicines, they have made large contributions to human health and well-being. Although, many drugs that come from plants sources have generally been replaced by more potent synthetic ones, some plants remain a source for some drug ingredient [1]. It is estimated by the World Health Organization that approximately 75-80% of the world's population use plant medicines either in part or entirely [2].

The main fraction of the global population in developing countries still relies on botanical drugs to meet its health requirements. The attention paid by health establishment to the use of herbal medicines has increased considerably, both because they are often the only medicine available in less developed areas and because they are becoming a popular alternative treatment in more developed areas [3]. Medicinal plants are frequently used as unprocessed materials for the extraction of active ingredients which is used in the synthesis of different drugs [4].

With the advent of ever-increasing resistant bacteria and yeast strains, there has been an equivalent rise in the universal demand for natural antimicrobial therapeutics that may constitute a reservoir of new antimicrobial substances to be discovered [5]. Recently, due to beneficial effects of antioxidants, particularly natural antioxidants, in the treatment and prevention of diseases, there has been an extensive interest in the discovery of natural antioxidants from plant sources. The studies on medicinal plants show that most of them possess significant antioxidant activity [6,7].

Beetroot (*Beta Vulgaris*) belongs to the Chenopodiaceae family and is initially from temperate climate regions of Europe and North Africa [8]. The red beetroot (*Beta vulgaris* ranks among the ten most potent vegetables with respect to antioxidant property. It makes an excellent dietary enhancement being not only rich in minerals, nutrients and vitamins but also has exceptional phyto constituents, which have several therapeutic properties [9]. Red Beet Betalain pigment also comprises an excellent natural food colorant and is effective against the oxidative stress and act as a scavenger of the free radical and reactive oxygen species like superoxide radical ion, singlet oxygen, hydroxyl radical, hydrogen peroxide, etc. which are associated with many diseases [10]. However, there is paucity of information on the antibacterial activity of crude extracts of *Beta vulgaris* against *Salmonella typhi, Klebsiella pneumonia* and *Citrobacter koseri* therefore; this study was designed to investigate the antimicrobial activity of crude extracts of *Beta vulgaris* (Beetroot) against these microbial isolates.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out at the Department of Biological Sciences, Bingham University. The University is located in AutaBalefi town, Karu Nasarawa State and has a tropical climate with two seasons: rainy and dry seasons. The University covers a land mass of 200 square meters and is geographically located at latitude 8.9565° N, 7.6997° E. [11].

2.2 Sample Collection

The *Beta vulgaris* plant was obtained from GaddaBiu market Jos, Plateau state, Nigeria. The plant was identified by experts in the Biological Sciences Department, Bingham University. The following are the voucher specimen number; SS138 and Herbarium specimen number; 27544 respectively. The bacterial isolates were obtained from Zanklin Research Centre, Bingham University.

2.3 Preparation of Extracts

The Bulb was weighed into three portions and each portion was used to produce different extract.

2.3.1 Maceration

The plant material; bulb were washed to remove debris and washed with distilled water. The fresh bulbs were used. The bulbs were measured (40 g) mashed, and inserted in a container with a solvent (200 ml Distilled Water) and allowed to stand at room temperature for 3 days with frequent agitation. The mixture was filtered using the Whatman's no 1 filter paper [12].

2.3.2 Boiling

The plant material (bulb) was measured (40 g), mashed and placed in a container containing 200 ml of distilled water. The mixture was boiled for 30 minutes with constant stirring. The mixture was allowed to cool and it was filtered using Whatman's no 1 filter paper.

2.3.3 Soaking

The fresh cleaned bulb was used. The bulb was measured (20 g), sliced, soaked in 100 ml of solvent (Distilled water contained) in a 500 ml sterile conical flask and covered with cotton wool. It was then plugged and wrapped with aluminium foil and shaken vigorously. The mixture was left to stand overnight (24 h) in a shaking water bath maintained at 40°C. The mixture was then filtered using a Whatman's No. 1 filter paper.

2.4 Determination of Antimicrobial Activity

Nutrient agar was prepared according to the manufacturer's instructions. Standard micro-biological procedures were adhered to. The test organisms were streaked onto the surface of the prepared nutrient agar medium in Petri dishes. The inoculated plates were allowed to stand for 30 minutes at room temperature for the organisms to pre-diffuse. After which, the inoculated plates were punched with a 5 mm cork borer to make wells in the agar plate. The wells were each filled with the different plant extracts aseptically after which, the plates were incubated at 37°C for 24 hrs. The antibacterial activity of the active constituents of the plant extracts on each of the test organism was determined by measuring inhibition zone diameter (IZD) in millimetres (mm).

3. RESULTS

Table 1 shows the mean zones of inhibition (mm) observed upon the introduction of crude extracts of *Beta vulgaris* to bacterial isolates. The highest mean zone of inhibition obtained was 6.67 mm from application of boiled crude extracts of *Beta vulgaris* to *Citrobacter koseri*. The lowest zone of inhibition observed was 0.3 mm upon the introduction of crude extract of *Beta vulgaris* to *Citrobacter koseri.*

Table 2 shows the mean zones of inhibition of bacterial isolates upon the application of antibiotics. The highest mean zone of inhibition of antibiotics was 43.67 mm observed from ciprofloxacin upon its application on *S. typhi.* The lowest mean zone of inhibition was 23.00 mm obtained from gentamicin upon its introduction on *C. koseri.*

Table 3 shows the mean zones of inhibitions of the crude extracts and control antibiotics, the highest mean zones of inhibition was 40.33 mm observed from ciprofloxacin upon its application

Table 1. Mean zones of Inhibition (mm) on of *S. typhi, K. pneumonia,* and *C. koseri* isolates

Test organism	Crude extracts			
	Intermediate	Boiled	Soaked	Macerated
Citrobacter koseri	0.3	6.67	0.00	0.00
Salmonella typhi	3.92	0.00	4.33	0.00
Klebsiella pneumonia	5.25	0.00	3.50	3.07

Table 2. Mean Zones of inhibition (mm) on isolates of *S. typhi, K. pneumonia,* and *C. koseri* upon the application of crude extracts

Test organisms	Antibiotics	Extracts			
		Intermediate	Boiled	Soaked	Macerated
Citrobacter koseri	Gentamicin	25.67	23.33	23.33	23.00
Salmonella typhi	Ciprofloxacin	35.00	41.33	41.33	43.67
Klebsiella pneumonia	Ampicillin	35.00	37.00	36.33	35.33

Table 3. Mean Zones of Inhibition (mm) on isolates of *S. typhi, K. pneumonia,* and *C. koseri* upon the application of crude extracts of *Beta vulgaris* and antibiotics

Test organisms & antibiotics	Extracts				
	Intermediate	Boiled	Soaked	Macerated	Antibiotics
Citrobacter koseri	0.30	6.67	0.00	0.00	26.08
Salmonella typhi	3.92	0.00	4.33	0.00	40.33
Klebsiella pneumonia	5.25	0.00	3.50	3.07	35.92

on *S. typhi* and the lowest mean zones of inhibition 3.07 mm obtained is upon the application of Intermediate extract on *C. koseri.*

4. DISCUSSION

In this study, the antibacterial activity of the crude extracts of *Beta vulgaris* was evaluated against *S. typhi, K. pneumonia* and *C. koseri* isolates. The crude extracts in different forms inhibited the growth of the microorganisms used in this study. This, however, disagrees with the findings of Koockak et al. [13] who tested the antibacterial activity of ethanolic extract of *Beta vulgaris* L. and reported that the extract did not possess inhibitory activity.

The boiled crude extract inhibited the test organism *C. koseri.* This may be due to the application of heat to the point of boiling; thereby vital phytochemicals were released from the plant bulbs. The soaked crude extract inhibited the test organisms *S. typhi,* and *K. pneumonia,* however, had no effect against *C. koseri*. The macerated crude extract inhibited the test organism *K. pneumonia,* and had no effect against the test organisms *S. typhi,* and *C. koseri.* This is similar to the findings of Čanadanović-Brunet et al. [14] and Sheila et al. [15] who reported antimicrobial activity of *Beta vulgaris* peel on *K. pneumonia.*

In separate studies, Rauha et al. [16] and Parekh and Chanda [17] tested the antimicrobial activity of methanolic extract of *Beta vulgaris* L. and aqueous leaf extract of *Beta vulgaris* L. They observed only slight antibacterial activity against the test organisms. The statistical analysis of the mean values (ANOVA) indicated there is no significant difference in the effect of the crude extracts of *Beta vulgaris* on the tests organisms.

The zones of inhibition for each of the controls used for the tests organisms in the antibacterial assay are presented. Ciprofloxacin produced the highest zone of inhibition on the test organism *S. typhi* while Gentamicin had the lowest zone of inhibition on the test organism *C. koseri*. The results from the table indicated that the tests organisms were susceptible to the antibiotics used against them. There was no significant difference in the application of antibiotics against the organisms.

There was significant difference between the zones of inhibition produced by the controls/antibiotic and *Beta vulgaris* extracts (at $p<0.05$, F Cal= 44.10 F tab= 3.48) and thus a longer usage of the crude extracts of *Beta vulgaris* will be required for a longer period of time for their effect to be achieved or an additive may be included in the extraction process (as indicated in the intermediate extract; lime). The *Beta vulgaris* crude extracts have little effect in a short period of time and require longer durations for their effects to be achieved. The process used in this study were crude methods and thus the use of more complex extraction methods may result in the extraction of more active compounds of the *Beta vulgaris.*

5. CONCLUSION

This study has shown the antibacterial potential of *Beta vulgaris* crude extracts against some microbial isolates. Crude extracts of *Beta vulgaris* has active components that can inhibit the growth of some microorganisms that cause infection. Further studies using conventional extraction techniques are recommended. This information can be useful in determining new antibacterial drugs. Hence there is a need for collaborative research between traditional herbal practitioners and modern scientist to develop novel antibacterial drugs.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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