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Antimicrobial and Antifungal Activity of Three Selected Homegrown Vegetables Consumed in Rwanda

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Authors' contributions

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

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ABSTRACT

This particular study is focused to the employment of herbal medicine due to the economical stringency of rural-based communities' resort of traditional medicine such as using *Moringa oleifera, Solanum nigrum* and *Cleome gynandra* as alternative drug sources to treat microbial infections without scientific knowledge about them. Therefore, the objective of this study was to determine the antibacterial activity of *Moringa oleifera, Solanum nigrum* and *Cleome gynandra* extracts with their extracted herbal juice. The aqueous solution of methanol, hexane and ethyl acetate extracts of *Moringa oleifera, Solanum nigrum* and *Cleome gynandra* leaves can be used for antimicrobial activity of such medicinal substitutes antibiotics like erythromycin, chloramphenicol, minocycline, and ketoconazole used as controls. The extracts (0.2 mg/ml) concentrations revealed varied antimicrobial activity against *Salmonella typhi, Escherichia coli, Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aerugenosa* bacteria as well as *Sacharomyces cervisiae* yeast

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and *Aspergillus fusarium* mold with respect to the inhibition diameters observed and measured on the agar. The maximal antibacterial activity of *Moringa oleifera* was observed against *Staphylococcus aureus* with fresh leaf aqueous extract (26.0 mm); *Solanum nigrum* against *Escherichia coli* with hexane extracts (22.0 mm), whereas the maximal antibacterial activity of *Cleome gynandra* was seen against *Salmonella typhi* with methanol extracts (19.0 mm). All the extracts from the three vegetables showed a higher antibacterial activity than the control except for *Pseudomonas aerugenosa.* The maximal antifungal activity of *Moringa oleifera* and *Solanum nigrum* was seen against *Sacharomyces cervisiae* with fresh leaf aqueous extracts (22.0 mm) while the maximal antifungal activity of *Cleome gynandra* was seen against *Sacharomyces cervisiae* with methanol extracts (21.0 mm).

Keywords: Homegrown vegetables; antimicrobial; nutrients; medicinal plants; edible vegetables.

ABBREVIATIONS

CST-UR	:	*College of Science and Technology, University of Rwanda (CST-UR)*
CHUK	:	(*Centre Hospitalier Universitaire de Kigal); University Teaching Hospital of Kigali*
B. subtilis	:	*Bacillus subtilis*
E. coli	:	*Escherichia coli*
S. aureus	:	*Staphylococcus aureus*
S. cerevisiae	:	*Saccharomyces cerevisiae*
P. aerugenosa	:	*Pseudomonas aerugenosa*
M. oleifera	:	*Moringa oleifera*
C. gynandra	:	*Cleome gynandra*
S. nigrum	:	*Solanum nigrum*
S. typhi	:	*Salmonella typhi*
A. fusarium	:	*Aspergillus fusarium*

1. INTRODUCTION

In the past, many African communities, particularly in Eastern African countries, Rwanda included have adopted ways to survive on wild edible vegetables not only in supplementing micronutrients in the diet but also they were sources for traditional medicinal practices [1]. Many kinds of literature have shown how Edible wild leafy vegetables played an important role in African agricultural nutritional and medicinal systems [2–5]. Despite such significant characteristics and properties provided, they have progressively disappeared while people came in contact with exotic products especially the western lifestyles and modernization [6–9].

Consequently in the international scene received little attention in term of research and development programs. It is only in the recent years that African that such vegetables and fruits get interested in public when politicians encouraging people to look back on this neglected reservoirs of food, feed as well as Medicine in the perspectives of mitigating growing malnutrition and imbalanced food. In Rwanda, as people are re-realizing the valuable role of such edible plants, there is hope that in near future days people will start to be interested on these plants diversity to be researched for increasing public awareness of using them not only for food and feed but medicines resources as well. It is estimated that there are more than 210 species of traditional leafy vegetables growing naturally in Rwanda [10–12] but not all have been fully utilized [13,14] in term of underpinning the valuable role in diet and phytochemical health related [15]. So far the edible plant species that are most used widely are *solanum nigrum, Cleome gynandra, Amaranthus sp*. among others some of which are well-known household medicines. The literature showed how *Solanum nigrum* leaves were and currently used to treat many diseases [16–18]. While leaves of *Cleome gynandra* may be crushed to make a concoction that is drunk to cure diseases such as scurvy; and is recommended for pregnant and lactating women to reduce dizziness. Likewise, elsewhere in Africa whereby plants have been extensively exploited for the treatment of many infectious diseases, Rwandans as well have been traditionally using plants for therapy [19–22]. This is due to that plants readily synthesize primary and second metabolites substances helping them to defend themselves against any attack

[23]. These substances are usually distributed in all plant's parts like roots, leaves, shoot and bark. Reasons why they become sources of active compounds necessary for drugs and other pharmaceutical products development. Recently public health consciousness focused on use of natural materials in the control of various infections looking side effects chemically synthesized products human health [24]. Unlike in developed countries whereby everything is documented filed away for next usage, many indigenous knowledgeable natural products with beneficial effects handed down by word of mouth and may get lost or distorted, consequently biological diversity be affected [6,25] Furthermore, as many communities in Rwanda cannot afford modern ways of harvesting, extracting and preserving, thus they still prefer to use old techniques of preservation, namely sprinkling with extracts from plant's parts. Unfortunately, such methods of doing things are restricted to home uses and could not be adapted for large-scale production. The lack of supporting systems for harvesting, processing and preserving definitely force them, for instance, to sell at low prices and/or consume the yield rapidly to prevent spoilage and losses. In light of the foregoing, there is a need for economic exploitation of the natural resources available to contribute to agricultural growth with concomitant reduction of poverty and increased sustainability of the livelihoods of the affected communities.

This research was therefore aimed at assessing the antimicrobial properties of the commonly consumed traditional vegetables in Rwanda (*solanum nigrum, Cleome gynandra, Amaranthus sp.*) which might be in future a possible source of novel antibiotics as currently some strains of bacteria are resisting to currently existing antibiotics.

2. MATERIALS AND METHODS

2.1 Collection of Plant Samples

The plant leafy parts of the selected plants; *Moringa oleifera, Solanum nigrum* and *Cleome gynandra* were collected from Musanze district, Nord province- Rwanda in July, 2015 and a taxonomist authenticated the samples.

2.2 Preparation and Isolation of Extract

The fresh leaves of *Moringa oleifera, Solanum nigrum* and *Cleome gynandra* (2984 gms, 2660 gms and 2360 mgs respectively). The leaves were then air-dried for two weeks under shade, followed by grinding into powder using the grinding mill. The powdered plant materials were extracted using maceration method [26] with minor modification, Solvents of differing polarity were then used separately, boiling water, hexane, methanol and ethyl acetate for 48 hours. The extracts obtained was cooled, micro filtered and pH was measured using the pH meter in order to evaluate the effect of solvents on microorganisms as the acidic pH may enhance the activity of plant extracts [27,28]. The extracts were concentrated using a rotary evaporator in vacuum at low temperatures 45ºC to avoid induction of oxidation and reduction reactions which might occur due to different conditions to give different grams of extracts as reported in Table 1; After the yield was calculated using below formula. This procedure was repeated three times for each plant and for each of the five solvents.

$$\text{Percentage yield } \left(\frac{\text{W}}{\text{v}}\right) = \frac{\text{Weight of Extract Recoverd}}{\text{Weight of Plant Sample}} \text{X } 100$$

2.3 Antimicrobial Activity

Preparation of 0.2 mg/ml extracts and controls for antimicrobial activity was carried out. by dissolving 2mgs of each extract in 10ml of solvent similarly to the control preparation. The crude extracts were assayed for antimicrobial activity against *Salmonella typhi, Escherichia coli, Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aerugenosa* bacteria as well as *Sacharomyces cervisiae* yeast and *Aspergillus fusarium* mould Disc diffusion method for antimicrobial susceptibility testing was carried out according to the standard method [29]. Pure isolates of the test microorganisms were obtained from Rwanda Medical Research Institute (CHUK microbiology Laboratory) with exactly 0.1 ml of cultures of each organism inoculated. Sterile paper discs impregnated with 0.2 mg/ml concentrates of different extracts as well as controls were placed on the agar and incubated at appropriate temperatures 37°C for 24 hours. Erythromycin was used as a control against *B. subtilis and P.aerugenosa;* chloramphenicol against *S. typhi* and *E. coli;* minocycline against *S. aureus;* ketoconazole against *S. cerevisiae* and *A. fusarium* and the zones of inhibition were measured using a ruler. Descriptive statistics method was used for data analysis.

Table 1. Yield calculation from different solvent's extracts

Plants	Solvent used	Mass of powder extracted	Mass of extract obtained	Yield (%)
Moringa oleifera	Methanol	300 g	6 g	2
	Water	300 g	4 g	1.3
	Hexane	300 g	5.5 g	1.8
	Ethyl acetate	300 g	5.2 g	1.7
Solanum nigrum	Methanol	350 g	7 g	2
	Water	350 g	5.8 g	1.6
	Hexane	350 g	6.7 g	1.9
	Ethyl acetate	350 g	6.3 g	1.8
Cleome gynandra	Methanol	250 g	5 g	2
	Water	250 g	4 g	1.6
	Hexane	250 g	4.5 g	1.8
	Ethyl acetate	250 g	4.7 g	1.9

3. RESULTS AND DISCUSSION

The highest inhibition diameters observed in *M. oleifera* was in fresh leaf aqueous extract with 26.0 mm against *S. aureus* through the boiled leaf extract showed a lower antibacterial inhibition (15 mm) against the same micro-organism (Table 2).

Other extracts did not show any inhibition of *S. aureus* with the control showing an antibacterial inhibition diameter of 9.0 mm. *M. oleifera* extracts against *P. aerugenosa* showed a lower inhibition diameter than the control (21.0 mm) with boiled leaf and methanol extracts showing no inhibition. The methanol extract did not show any antibacterial inhibition on the test microorganisms (Table 2).

M. oleifera fresh leaf extract showed a high antifungal activity against *S. cerevisiae and A. fusarium* (22.0 mm and 20.0 mm) in comparison to the control (15.0 mm and 14.0 mm) (Table 3).

The (Table 4) summarizes the results of the Disc diffusion assay of the leaves extracts from *S. nigrum* against *E. coli, S. subtilis, S. typhi* respectively; therefore the highest inhibition diameters from *S. nigrum* leaves extracts was in hexane solvent with 22.0 mm against *E. coli,* 21.0 mm against *S. subtilis* and 20.0mm against *S. typhi* (Table 4). The extract obtained using Ethyl acetate didn't show any activity against *S. aureus* and *B. subtilis* while methanol extracts could not show any activity against *P. aerugenosa* and *E. coli* respectively.

The higher antibacterial activity could be also observed against *P. aerugenosa* with (21.0 mm) compared with all extracts of the vegetable. Generally all the extracts could show higher antifungal activity than the controls and our results for *S. nigrum* aqueous extracts also coincide with other findings [30,31] as it was found that *S. nigrum* have higher antifungal activity (Table 5) than other solvent extracts with fresh leaf aqueous extract being the highest against *S. cerevisiae* (22.0mm) and *A. fusarium* (18.0 mm) accordingly.

In (Table 6), we summarize, the minimal antibacterial activity that was shown from *C. gynandra* leaves extracts against *S. aureus* and *B. subtilis* respectively.

Table 2. Bacterial inhibition diameters (mm) of *Moringa oleifera*

Extract	*S. typhi*	*P. aerugenosa*	*E. coli*	*S. aureus*	*B. subtillis*
Mfw (6.2)	21.0 ± 0.9	0.0 ± 0.0	22.0 ± 2.0	26.0 ± 2.0	17.0 ± 1.5
Mbw (7.4)	0.0 ± 0.0	20.0 ± 1.5	0.0 ± 0.0	15.0 ± 1.0	18.0 ± 1.0
Mm (5.5)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Mh (5.6)	19.0 ± 1.0	20.0 ± 2.0	20.0 ± 0.5	0.0 ± 0.0	0.0 ± 0.0
Mea (5.6)	17.0 ± 0.5	0.0 ± 0.0	22.0 ± 2.0	0.0 ± 0.0	0.0 ± 0.0
Control	13.0 ± 0.8	21.0 ± 1.0	9.0 ± 0.5	9.0 ± 1.5	15.0 ± 2.0

M- Moringa; fw- fresh leaf water; bw-boiled leaf water; m- methanol; h- hexane; ea- ethyl acetate; Values in brackets are pH values of extracts

Table 3. Fungal inhibition diameters (mm) of *Moringa oleifera*

Extract	*S. cerevisae*	*A. fusarium*
Mfw (6.2)	22.0 ± 2.0	20.0 ± 1.0
Mbw (7.4)	21.0 ± 1.0	19.0 ± 2.0
Mm (5.5)	17.0 ± 1.0	15.0 ± 1.0
Mh (5.6)	0.0 ± 0.0	0.0 ± 0.0
Mea (5.6)	0 .0 ± 0.0	0.0 ± 0.0
Control	15.0 ± 1.0	14.0 ± 1.0

M- Moringa; fw- fresh leaf water; bw-boiled leaf water; m- methanol; h- hexane; ea- ethyl acetate; Values in brackets are pH values of extracts

The extracts from leaves that were fresh and boiled exhibited minimal activity against all the tested bacteria, even though shown meaningful antifungal activity; thus their following zones of inhibition could be observed respectively *S. cerevisiae* (16.0 mm) and *A. fusarium* (15.0 mm) (Table 7). Finally, the *C. gynandra* methanol extract was found to have the highest antifungal activity against *S. cerevisiae* (21.0 mm) and *A. fusarium* (17.0 mm), these results corresponded with other findings [32].

The growth of *P. aeruginosa* was inhibited by an aqueous crude extract of *Solanum nigrum* both fresh leaf extract and boiled extract. As reported by [33] it is obvious that aqueous extracts are generally richest in antibacterial agents; on top of that, water had been reported by traditional healers and herbalists to be the most commonly used solvent to extract biologically active compounds evidently due to its easy availability [24,34]. However, these results revealed that other methods and techniques may be applied and be compared for their efficiency as not always water is the most effective solvent pharmacologically active compounds extraction in tested plants. Even in this study a better understanding to unravel and shed light on antimicrobial active compounds with their mode of action may be difficult to speculate; moreover some people proposed that many antimicrobial agents may exhibit their action through inhibition and disruption of many cellular processes such as nucleic acid, protein and membrane phospholipids biosynthesis [35,36]. Possibly the antimicrobial agent(s) in the extracts inhibit microbes one way or another via some of the above mechanisms. Various chemical constituents previously reported being isolated from *Solanum* species, which includes alkaloids, phenolics, flavanoides, sterols saponins and their glycosides probably confer the antimicrobial activity of the plants [10,37]. The results of the antibacterial activities done by [38,39] showed that the plant's extract presented remarkable activity against the test organisms *E. coli*, *K. pneumonia*, *P. aeruginosa*, *S. aureus* with zones of inhibition which are slightly greater than the results we obtained in this work, probably due to

Table 4. Bacterial inhibition diameters (mm) of *Solanum nigrum*

Extract	*S. typhi*	*P. aerugenosa*	*E. coli*	*S. aureus*	*B. subtillis*
Sfw (6.3)	19.0 ± 1.0	16.0 ± 2.0	21.0 ± 2.0	15.0 ± 1.0	19.0 ± 1.0
Sbw (6.4)	0 .0 ± 0.0	18.0 ± 1.5	18.0 ± 1.0	19.5 ± 1.5	0.0 ± 0.0
Sm (6.2)	21.0 ± 2.0	0.0 ± 0.0	0.0 ± 0.0	18.0 ± 2.0	18.0 ± 2.0
Sh (5.5)	20.0 ± 1.0	19.0 ± 2.0	22.0 ± 2.0	16.0 ± 1.0	21.0 ± 0.0
Sea (6.6)	17.0 ± 1.0	15.0 ± 1.0	18.0 ± 1.5	0.0 ± 0.0	0.0 ± 0.0
Control	13.0 ± 1.0	21.0 ± 1.0	9.0 ± 1.0	9.0 ± 2.0	15.0 ± 1.0

S- Solanum; fw- fresh leaf water; bw-boiled leaf water; m- methanol; h- hexane; ea- ethyl acetate; Values in brackets are pH values of extracts

Table 5. Fungal inhibition diameters (mm) of *Solanum nigrum*

Extract	*S. cerevisae*	*A. fusarium*
Sfw (6.3)	22.0 ± 2.0	18.0 ± 2.0
Sbw (6.4)	21.0 ± 1.0	17.0 ± 1.0
Sm (6.2)	19.0 ± 1.0	16.0 ± 1.0
Sh (5.5)	18.0 ± 2.0	17.0 ± 2.0
Sea (6.6)	0.0 ± 0.0	0.0 ± 0.0
Control	15.0 ± 1.0	14.0 ± 1.0

S- Solanum; fw- fresh leaf water; bw-boiled leaf water; m- methanol; h- hexane; ea- ethyl acetate; Values in brackets are pH values of extracts

Table 6. Bacterial inhibition diameters (mm) of *Cleome gynandra*

Extract	*S. typhi*	*P. aerugenosa*	*E. coli*	*S. aureus*	*B. subtillis*
Cfw (8.0)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Cbw (5.7)	0.0 ± 0.0	0.0 ± 0.0	15.0 ± 1.0	0.0 ± 0.0	0.0 ± 0.0
Cm (6.3)	19.0 ± 2.0	15.0 ± 2.0	19.0 ± 1.0	0.0 ± 0.0	0.0 ± 0.0
Ch (6.7)	15.0 ± 1.0	15.0 ± 1.0	16.0 ± 2.0	0.0 ± 0.0	0.0 ± 0.0
Cea (6.7)	1.0 ± 0.5	15.0 ± 1.0	18.0 ± 1.0	0.0 ± 0.0	0.0 ± 0.0
Control	13.0 ± 1.0	21.0 ± 2.0	9.0 ± 2.0	9.0 ± 2.0	15.0 ± 1.5

C- Cleome; fw- fresh leaf water; bw-boiled leaf water; m- methanol; h- hexane; ea- ethyl acetate

differences in concentration of the plants extracts used. Our invigations revealed that the plant's extracts from *Moringa oleifera*, *S. nigrum* and *cleome gynandra* were active against the tested fungal, gram positive and gram negative bacteria and these results suggested their use in traditional medicine.

Table 7. Fungal inhibition diameters (mm) of *Cleome gynandra*

Extract	*S. cerevisae*	*A. fusarium*
Cfw (8.0)	16.0 ± 2.0	15.0 ± 1.5
Cbw (5.7)	16.0 ± 1.5	15.0 ± 1.0
Cm (6.3)	21.0 ± 2.5	17.0 ± 2.0
Ch (6.7)	0 .0 ± 0.0	0.0 ± 0.0
Cea (6.7)	0.0 ± 0.0	0.0 ± 0.0
Control	15.0 ± 1.0	14.0 ± 1.0

C- Cleome; fw- fresh leaf water; bw-boiled leaf water; m- methanol; h- hexane; ea- ethyl acetate; Values in brackets are pH values of extracts

4. CONCLUSION

This research shows that apart from being nutritionally beneficial, these indigenous vegetables have antimicrobial properties that are beneficial for human health. The antimicrobial activity of most of the extracts against the test microorganisms qualifies these plants for further investigation of their bioactive compounds. Further studies on the *in vivo* activity, isolation and structural elucidation of the active component(s) of the plant extract are therefore recommended to comprehensively tap their potential.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

AVAILABILITY OF DATA AND MATERIALS

The datasets supporting the conclusions of this article are included within the article.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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