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University of Eldoret, Kenya, Mount Kenya University, Kenya, Chukwuemeka Odumegwu Ojukwu University, Nigeria, Kyambogo University, Uganda and University of Makeni, Sierra Leone

# Ripening-Dependent Antimicrobial Activity of African Nightshade Berry Extracts: Implications for Food Preservation and Pharmacology

***Heka Kamau[1]**
*[1]Department of Food, Nutrition & Consumer Sciences, University of Eldoret*

**Corresponding email: ekellyne@gmail.com*

## Abstract
*Evidence of bioactive compounds in African nightshade (Solanum nigrum Complex) edible berries necessitated an investigation into the antimicrobial activity of edible berry extracts from four African nightshade (Solanum nigrum complex) varieties at different stages of ripening. Ethanolic extracts were prepared from Black NS, Giant NS, JKUAT Improved, and KALRO Agriculture varieties harvested at green, colour break, ripe, and senescence stages. The extracts were tested against Gram-positive bacteria (Staphylococcus aureus and Bacillus cereus), Gram-negative bacteria (Escherichia coli and Salmonella typhi), and fungi (Candida albicans and Aspergillus niger) using the hole-plate diffusion method, while minimum inhibitory concentration (MIC) assays were used to determine extract potency. Results showed that antimicrobial activity varied significantly with variety, ripening stage, and microorganism type. Black NS and KALRO Agriculture varieties demonstrated the highest inhibitory effects, particularly at the green and colour break stages. Gram-positive bacteria were more susceptible to the extracts than Gram-negative bacteria, with S. aureus recording the greatest sensitivity. Fungal species exhibited the least susceptibility, requiring higher concentrations for inhibition. Antimicrobial potency decreased progressively with ripening, likely due to reductions in bioactive compounds such as phenolics, tannins, flavonoids, and alkaloids. MIC values ranged from 6.25–12.50 µg/mL for most bacterial species, while fungal inhibition required concentrations of 100 µg/mL. The findings demonstrate that African nightshade berries possess significant natural antimicrobial properties and could serve as potential sources of plant-based preservatives and medicinal ingredients for the food and pharmaceutical industries.*

***Key words**: African nightshade, Extract, Antimicrobial Activity, Berries*

## INTRODUCTION
Whilst evidence has it that there are phytochemicals in the *S. nigrum*, there is need to prove that these are actually active against micro-organisms. Studies have been done in attempt to ascertain this. Matasyoh et al. (2014) noted that traditionally, these properties of the *S. nigrum* were appreciated and this is the reason as to why they are used as traditional medicine. For instance, in Kenya, the unripe fruits are used in soothing tooth pain and are also squeezed on teething babies' gums to ease the pain (Matasyo *et al*., 2014). In other places, the leaves are used as a remedy for stomach ache while the extracts from both the leaves and the fruits are used in treating tonsillitis (Modilal *et al*., 2015). As such, the plant finds use as a replacement for synthetic drugs which could be quite expensive yet they could have side effects.

Puupponen-Pimia *et al*. (2001) concluded that the antimicrobial effect of berries is dependent on the species as well as type of microorganism studied. Although other berries have been extensively screened for their anti-microbial activity, little has been done on the berries of the African nightshade. Much attention has been on the leafy part of the crop, neglecting the potential of the berries as a food item. Consequently, there is minimal evidence to support their use as a food item or any application in food processing and preservation. This study aimed at filling this knowledge gap by screening for the antimicrobial properties of the berries.

## MATERIALS AND METHODS
### Sampling and Preparation of Plant Samples
Sixteen (16) samples were screened for their anti-microbial activity. These were samples from four African nightshade varieties; Giant NS, Black NS, JKUAT Improved and KALRO Agriculture varieties. The berries were harvested at the four stages of ripening; green, colour break, ripe and senescence. The samples were oven-dried till they attained a constant weight. This was followed by pulverization in a super mixer grinder and then packed in sterile paper bags. These were then stored at room temperature (25º C) awaiting further tests (Matasyoh *et al*., 2014).

**Crude Extract Preparation**

This was done as described by Mostafa *et al*. (2018) and Liepiņa *et al*. (2013) with modifications. About 10 g of the powdered sample was soaked in 40 ml of ethanol for 2 hours. The macerated material was then ground using a mortar and pestle before being mixed with an extra 40 ml of ethanol. This was extracted for a further 10 hours and then filtered through double layer of muslin cloth. These were then centrifuged for 10 minutes at 9000 rpm and filtered using Whatman filter paper No. 41 to obtain a clear filtrate. The filtrates were then evaporated and dried at 40° C then stored in closed bottles awaiting further tests. The concentration of the final extract was 0.2 mg/mL. The extracts were weighed and yield percentages calculated as follows:

$$yield\% = \frac{R}{S} x\ 100$$

Where R = weight of extracted plant residues and S = weight of raw sample (Mostafa *et al*., 2018).

**Test Microbes**

Five microbes were used in the anti-microbial tests. These included Gram positive bacteria *Staphylococcus aureus* (ATCC 25923) and *Bacillus cereus* (ATCC 11778) as well as Gram negative bacteria *Escherichia coli* (ATCC 25922) and *Salmonella typhi* (ATCC 13347). *Candida albicans* (ATCC 90028) and *Aspergillus ni*ger (ATCC 16404) were the fungi species used. These strains were obtained from the microbiology laboratory at the University of Eldoret.

**Antimicrobial Sensitivity Tests**

The hole-plate diffusion method as explained by Abbas *et al*. (2014) was used to test for the bacterial and fungal sensitivity to the extracts of the African nightshade berries at different ripening stages. The method was applied with modifications (Rojas *et al*., 2006; Matasyoh *et al*., 2014; Mostafa *et al*., 2018). To prepare the inoculum, a suitable suspension of each Gram negative and Gram positive bacteria was prepared by incorporating a loop of fresh micro-organism in 10 ml sterilized water. This was evenly mixed in preparation for injection near flame.

For each Gram positive and Gram negative bacteria, about 10 ml of the prepared inoculum was poured into 500 ml of sterile nutrient agar medium. This was used for the determination of the anti-bacterial activity. For the anti-fungal activity, 10 ml of each of the fungal inoculum that was previously prepared was poured into 500 ml of sterile liquified potato dextrose agar (PDA) (Madhuri and Sarasamma, 2019). After carefully labelling the media for each micro-organism, the media were gently shaken to allow for uniform mixing of the media and the inoculum.

After sufficient mixing, the media were poured into labelled, sterile petri-dishes to a specified quantity such that the depth was maintained at about 8 mm. This was achieved while gently rotating the petri-dishes and evenly spreading the media as it was allowed to solidify at room temperature. Four holes were made in the solidified medium using a stainless steel borer, making sure that the holes were equidistant from each other. The holes were labelled numbers 1 to 4. Hole 1 was filled with the positive control reference; Ampicillin (Crown Healthcare, Nairobi, Kenya) at dose of 125 mg/mL for bacterial assays and Amphotericin B (Health Biotech Ltd., India) at a standard dose of 5 mg/mL for fungal assays (Abbas *et al*., 2014). Holes 2-4 were filled with sample solution from each of the *S. nigrum* complex extracts at 20 mg/ml. This was done for each variety at each ripening stage. For testing the anti-bacterial activity, the petri-dishes were incubated at 37 ˚C for 48 hours while for the anti-fungal activity, the incubation was carried out for 72 hours. The antimicrobial activity was tested by measuring the diameters of the zones of inhibition in millimeters using a clear ruler. The results were then expressed as the mean zones of inhibition.

**Minimum Inhibition Concentration**

From the anti-microbial assay, it was discovered that the green stage of ripening was the most potent against the micro-organisms. As such, the extracts at the green stage of each variety of African nightshade was used for the MIC tests. This was carried out using the method described by Seleshe *et al*. (2017) with modifications. Six (6) vials in total were used for each extract. The first vial was filled with 100 µL of the extract from a stock solution while the 2$^{nd}$ to 6$^{th}$ vials were filled with 50 µL of sterile water. Serial dilution was then carried out from 2$^{nd}$ to 6$^{th}$ vials and this was achieved by drawing and transferring 50 µL from the first vials to the subsequent vials to obtain serially descending concentrations of 100, 50, 25, 12.5, 6.25 and 3.12 µg/ml.

A positive control was used where the microorganism was added in the vial with no extract while negative control contained the extract but no micro-organism. 20 µL of the of the bacterial and fungal suspensions were added to each vial and then incubated at 37 ˚C for 24 hours (bacterial) and 48 hours (fungi) as shown by Seleshe *et al*. (2017). After the incubation period, 80 µL of resazurin dye was added before the samples were re-incubated for a further 2 hours to

facilitate colour development. Inhibition was indicated by the blue coloration of the wells after the addition of the dye. Colour change from blue to red was an indication of the presence of live micro-organisms and, therefore, an indication that inhibition did not take place.

## RESULTS AND DISCUSSIONS

### Extraction Yield

The extraction yield for the different berries is as reported in Table 1.

**Table 1: Ethanolic Extraction Yield (%) of Different Varieties of African Nightshade Berries at Different Stages of Ripening**

| Variety | Ripening Stage | | | |
|---|---|---|---|---|
| | *Green* | *Colour Break* | *Ripe* | *Senescence* |
| Black N.S | 30.15 | 30.58 | 45.60 | 61.11 |
| Giant N.S | 28.21 | 31.12 | 50.11 | 59.22 |
| JKUAT Impr. | 29.32 | 33.21 | 48.90 | 62.00 |
| KALRO Agric. | 30.44 | 32.50 | 50.21 | 65.24 |

The berry yields ranged from a lowest of 28.21% in green Giant NS to a high of 65.24% in KALRO Agric. variety at senescence. A trend was observed where the berry yield increased as the berries ripened. This was observed amongst all berries, where the yield of the berries at senescence was, slightly higher than twice the yield at the green stage for all the varieties. The differences observed within each variety but at different ripening stages as well as amongst the various varieties could be attributed to the variations in type and amount of soluble compounds in the respective samples (Matasyoh *et al*., 2014). The yields also seemed to be similar in quantities amongst the different varieties at each particular stage. Similar trends were observed by Saleh and Otaibi (2013) who analysed the yield of dates at different ripening stages and (Mostafa *et al*., 2018).

The yields for the berries were higher than yields stated in previous studies of African nightshade leaves (Matasyoh *et al*., 2014). The ethanolic yield at the green and colour break stages were comparable to methanolic extracts of nightshade berries in a study carried out by Abbas *et al*. (2014). Nevertheless, it is notable that Abbas *et al.* (2014) did not specify at which stage of ripening they sampled from. In the current experiment, it was not possible to test the significant difference within and between samples because the extraction was carried out in batch for each variety and each ripening stage, therefore the statistical differences could not be calculated.

### Anti-Microbial Activity of Ethanolic Extracts of *Solanum nigrum complex* Edible Berries

The antimicrobial activity of the berries was determined using the inhibition zones and the results were as reported on Table 2. African nightshade edible berry extracts proved potent to enterocytic microbes. Inhibition of *E. coli* ranged from a high of 9.0 mm observed in the colour break stage of Black NS to a low of 5.0 mm recorded at the senescence stage of JKUAT Improved variety. This inhibition, however, was significantly lower than the 14.7 mm recorded by the positive control (ampicillin). In terms of varietal differences, it was noted that Black NS and KALRO Agriculture varieties recorded the highest inhibition against the microbe, followed by the Giant NS and lastly JKUAT improved variety. Inhibition of *S. typhi* was lower than inhibition of *E. coli*. The highest inhibition as recorded at the green and colour break stages of Black NS, with an inhibition zone of 8.2 mm. Inhibition values of 5.0 and 4.2 mm recorded at the senescence stages of Giant NS and JKUAT improved varieties, respectively, were the least values recorded. Once again, Black NS berry extracts were significantly more potent against this micro-organism, even though this was not statistically different from the values recorded for the KALRO variety. These two varieties, however, proved to be significantly more potent against *S. typhi* at all stages of ripening compared to Giant NS and JKUT Improved varieties. Action of the extracts seemed to be more inhibiting against Gram positive bacteria compared to Gram negative bacteria. *S. aureus*, a Gram negative bacteria, was the most sensitive to the berry extracts. It recorded a high inhibition zone value of 11.8 mm seen in Black NS at the colour break stage while 5.2 mm and 5.3 mm recorded at senescence stage of Giant NS and JKUAT Improved, respectively, were the lowest values. These, however, were still significantly higher than the values recorded for the Gram negative bacteria. Black NS and KALRO Agriculture varieties were superior to the rest in inhibition of *S. aureus*.

*B. cereus* is also a Gram positive bacteria. Although its inhibition was significantly lower than that of *S. aureus*, it was still significantly higher than the inhibition observed in both *E. coli* and *S. typhi*. Inhibition ranged from a high of 10.8

mm in the colour break stage of Black NS to 5.3 mm recorded in Giant NS at senescence. For all the bacterial assays, the African nightshade berry extracts showed inhibition capacity. However, the recorded values were still significantly lower than the values recorded in the positive controls. Although the extracts also exhibited antimicrobial activity against fungi, this was not comparable to the action against bacteria, indicating that the extracts would be less effective against fungal-related problems. Just like in the antibacterial assays, the extracts of the berries showed greater inhibition in the green and colour break stages. Indeed, no inhibition was recorded at the senescence stages of all the varieties while JKUAT improved also showed no inhibition potential at the ripe stage.

Inhibition of *C. albicans* was higher than inhibition of *A. niger* across all varieties. However, the trend observed in the bacterial assays was still evident. Black NS and KALRO Agriculture varieties had a higher inhibition effect at all stages compared to the Giant NS and JKUAT Improved varieties. The inhibition of *A. niger* was comparable between Black NS and KALRO Agriculture but with reference to *C. albicans*, KALRO Agriculture had a greater inhibitory effect.

**Table 2: The Antimicrobial Activities from Different Varieties of African Nightshade Berries against 6 Micro-Organisms**

| Variety/Stage | Diameter of Inhibition Zone (mm) | | | | | |
|---|---|---|---|---|---|---|
| | ***E. coli*** | ***S. typhi*** | ***S. aureus*** | ***B. cereus*** | ***C. albicans*** | ***A. niger*** |
| **Green** | | | | | | |
| Black NS | 8.8±0.3$^{gh}$ | 8.2±0.3$^{g}$ | 10.8±0.3$^{fg}$ | 10.2±0.3$^{i}$ | 5.3±0.6$^{gh}$ | 5.3±0.6$^{hij}$ |
| Giant NS | 7.7±0.6d$^{ef}$ | 7.7±0.3$^{fg}$ | 8.3±0.6$^{e}$ | 8.8±0.3$^{h}$ | 5.2±0.3$^{gh}$ | 4.5±0.5$^{ghij}$ |
| JKUAT Impr. | 6.7±0.6$^{bcd}$ | 5.3±0.6$^{abc}$ | 7.7±0.6$^{de}$ | 7.5±0.5$^{ef}$ | 4.8±0.3$^{fg}$ | 4.3±0.6$^{fghi}$ |
| KALRO Agric. | 8.2±0.3$^{fgh}$ | 7.7±0.6$^{fg}$ | 10.0±0.6$^{f}$ | 9.7±0.5$^{hi}$ | 6.3±0.6$^{h}$ | 5.7±0.6$^{ij}$ |
| **Colour Break** | | | | | | |
| Black NS | 9.0±0.0$^{h}$ | 8.2±0.3$^{g}$ | 11.8±0.3$^{g}$ | 10.8±0.3$^{i}$ | 5.7±0.3$^{gh}$ | 5.8±0.3$^{j}$ |
| Giant NS | 7.2±0.3$^{def}$ | 6.7±0.6$^{def}$ | 8.2±0.3$^{de}$ | 8.2±0.3$^{fg}$ | 4.3±0.6$^{efg}$ | 3.7±0.6$^{defg}$ |
| JKUAT Impr. | 6.0±0.0$^{abc}$ | 5±0.0$^{abc}$ | 7.5±0.5$^{cde}$ | 7.0±0.0$^{cdef}$ | 3.3±0.6$^{cde}$ | 3.0±0.0$^{cdef}$ |
| KALRO Agric. | 7.8±0.3$^{efg}$ | 7.2±0.3$^{efg}$ | 10.3±0.6$^{f}$ | 9.7±0.3$^{hi}$ | 5.5±0.5$^{h}$ | 5.0±0.0$^{ghij}$ |
| **Ripe** | | | | | | |
| Black NS | 7.0±0.0$^{cde}$ | 6.2±0.3$^{cde}$ | 8.2±0.3$^{de}$ | 7.3±0.6$^{def}$ | 2.0±0.0$^{b}$c | 2.3±0.6$^{bcd}$ |
| Giant NS | 5.8±0.3$^{ab}$ | 5.5±0.5$^{bcd}$ | 6.8±0.3$^{bcd}$ | 6.2±0.3$^{abcd}$ | 1.7±0.6$^{ab}$ | 1.3±0.6$^{ab}$ |
| JKUAT Impr. | 5.3±0.6$^{a}$ | 4.8±0.3$^{ab}$ | 5.8±0.3$^{ab}$ | 6.0±0.0$^{abc}$ | ND | ND |
| KALRO Agric. | 6.0±0.0$^{abc}$ | 5.8±0.3$^{bcd}$ | 6.8±0.3$^{bcd}$ | 6.7±0.6$^{cde}$ | 2.7±0.6$^{bcd}$ | 2.0±0.0$^{bc}$ |
| **Senescence** | | | | | | |
| Black NS | 6.8±0.3$^{bcde}$ | 5.8±0.3$^{bcd}$ | 7.3±0.6$^{cde}$ | 7.2±0.3$^{cdef}$ | ND | ND |
| Giant NS | 5.3±0.6$^{a}$ | 5.0±0.0$^{abc}$ | 5.3±0.6$^{a}$ | 5.3±0.6$^{a}$ | ND | ND |
| JKUAT Impr. | 5.0±0.0$^{a}$ | 4.2±0.3$^{a}$ | 5.2±0.3$^{a}$ | 5.5±0.5$^{ab}$ | ND | ND |
| KALRO Agric. | 5.8±0.3$^{ab}$ | 5.3±0.3$^{abc}$ | 6.2±0.3$^{abc}$ | 6.0±0.0$^{abc}$ | ND | ND |
| Ampicillin | 14.7±0.6$^{i}$ | 13.3±0.6$^{h}$ | 16.7±0.6$^{h}$ | 14.3±0.6$^{j}$ | ND | ND |
| Amphotericin B | ND | ND | ND | ND | 12.8$^{i}$ | 11.2$^{k}$ |

Values are mean± SD. Values followed by different letter superscripts in a column are significantly different at p≤0.05 as assessed by Tukey's least significant test
ND = Not detected

Plants contain antimicrobial substances to protect them from microbial infection and deterioration (Abbas *et al*., 2014). These are substances such as flavonoids and other polyphenolic compounds including alkaloids, lignins, tannins, glycosides (Puupponen-Pimia *et al*., 2001; Smith *et al*., 2005). They also contain terpenoids such as monoterpenes, diterpenes or triterpenes and sesquiterpenes (Abbas *et al*., 2014). The mode of antimicrobial activity can be attributed to these compounds getting through the bacterial and fungal cell walls or membranes and suppressing the growth of the microbes. If the compounds penetrate deeply, they could lead to the death of the micro-organisms (Abbas *et al*., 2014). Smith *et al*. (2005) propose a mechanism by which this inhibition occurs whereby the tannins in plants complex with minerals and polymers. Consequently, the tannins lead to inhibition of extracellular microbial enzymes, deprivation of the substrates needed for microbial growth as well as direct impact on the microbial metabolism through iron deprivation or inhibition of oxidative phosphorylation (Scalbert, 1991; Nohynek *et al*., 2006). However, some gastrointestinal bacteria can override this mechanism through mechanisms such as tannin modification, or degradation, dissociation of the tannin-substrate complexes, membrane modification or repair and tannin inactivation by high-affinity binders (Abbas *et al*., 2014). This could explain why the inhibition varies depending on the micro-organisms since not all can overcome the inhibition effect of the polyphenols (Bobinaitė *et al*., 2013; Puupponen-Pimia *et al*., 2005).

The results of this study on antimicrobial effect of the African nightshade berries are corroborated by previous works using berries. In evaluating the antimicrobial activity of *Solanum nigrum* using ethyl acetate extracts, Abbas *et al*. (2014) found inhibition zones of 7.2 mm in *S. aureus*, 6.7 mm in *S. typhi*, 7.5 mm in *E. coli* and 6.7 in *C. albicans*. In a study to test efficacy of different berries with storage time on *E. coli* E-564$^{T}$ and *Salmonella* sv. SH-5014, it was recorded that cloudberry and raspberry extracts led to the death of both cultures but cloudberry was more potent on *Salmonella* even after being frozen for 12 months. Bilberry showed clear inhibition on *E. coli* but strong inhibition on *Salmonella* while Black currant recorded weak inhibition on both microbes (Nohynek *et al*., 2006).

A study on antimicrobial properties of black chokeberries' extracts obtained from fresh, frozen and dried fruits exhibited inhibition against Gram negative bacterium *Pseudomonas aeruginosa* but did not have any effect on *E. coli* (Liepiņa *et al*., 2013). Radovanović *et al*. (2013) evaluated the antimicrobial activity of blackthorn, European cornel and wild blackberry against five Gram positive and five Gram negative bacteria and concluded that all the microbes were highly sensitive to all the extracts, though to varying degrees, displaying inhibition zones in the range of 12.0 - 16.2 mm using concentrations of 50 µl disc$^{-1}$. *S. enteridis* was the most sensitive amongst the Gram negative bacteria while *S. aureus* was most sensitive in Gram positive bacteria.

Similar findings were also reported by Seleshe *et al*. (2017) while evaluating antimicrobial activity of ethanolic extracts of three types of strawberries, exhibiting inhibition zones of 11.5 – 12.5 mm against *S. aureus*, 10.5 – 13.5 against *S. pneumonia*e and 8.5 – 10.5 against *E. coli* but with no inhibition activity against fungi *A. niger* and *C. albicans*, an observation supported by Bobinaitė *et al*. (2013). This study found out that fungi are less susceptible to the berry extracts compared to bacteria.

It was noted that across the varieties, the antimicrobial activity decreased as the berries ripened. As such, berries at the green stage were more potent against the test micro-organisms compared to all the other stages. This was with the exception of Black NS in which berries at the colour break stage had the highest zone of inhibition for the variety. However, this was not statistically different from the green stage of the same variety. Indeed, this trend was observed across all varieties where the antimicrobial activity did not seem to vary significantly from the green too the colour break stage. However, a significant drop in microbial activity was observed on transition from the colour break to the ripe stages and this drop was sustained through to senescence.

The observed decrease in antimicrobial activity upon ripening was reported in previous studies involving other fruits. For instance, Enemuor *et al*. (2011), when comparing the antimicrobial activity of ripe and unripe fruit extracts of *Cissus multistriata* concluded that unripe fruits were more potent to bacteria as compared to unripe fruits. A similar conclusion was also arrived at by Saleh and Otaibi (2013) upon evaluation of antimicrobial activity of dates at 3 different stages of ripening. A study by Dawkins *et al*. (2003), however, reported that anti-microbial activity of *Carica papaya* fruits extracts was independent of the ripening stage. This difference could be attributed to species differences in the samples used, but this could be open for further investigation.

This drop in potency upon ripening could be attributed to alteration of the bioactive components in the fruits, thereby leading to less inhibition (Enemuor *et al*., 2011). As demonstrated by Saleh and Otaibi (2013), polyphenols play a major role in anti-microbial activity through enzyme inhibition and precipitation of proteins. Other bioactive compounds such as tannins, flavonoids and alkaloids also have antimicrobial function as they serve to protect the plant from microbial spoilage ( Puupponen-Pimia *et al*., 2001; Abbas *et al*., 2014). Therefore, alteration of these compounds could lead to the decrease in the microbial inhibition action of the extracts. For this study, it was noted that the various phytochemicals in the African nightshade berries decreased as the berries ripened.

The quantity of total phenols, flavonoids and tannins all decreased with ripening as reported by Kamau *et al.,* (2020). Given that micro-organisms are sensitive to pH as this affects their enzyme activity (Saleh and Otaibi, 2013), it is possible that the decrease in titrable acidity of the berries as they ripened (Kamau *et al.,* 2020) could also have led to the decrease in microbial inhibiting in the latter stages of ripening. However, this is refuted by Liepiņa *et al*. (2013) upon investigation of antimicrobial activity of extracts from fruits of *Aronia melanocarpa* and *Sorbus aucuparia*. These researchers concluded that pH was not a determining factor for antimicrobial activity of extracts since there was no observed association between the pH value and antimicrobial activity. However, it is notable that Liepiņa *et al*. (2013) only considered one stage of fruit ripening in their study. As such, they could not compare the effect of decreasing pH as occasioned by fruit ripening as was evaluated in the current study. Conclusively, it can be deduced that decrease in acidity with ripening could have a negative impact on the microbial inhibition capacity of the African nightshade berries.

Berry extracts (including *S. nigrum*) have previously been reported to be more potent against Gram positive bacteria than Gram negative bacteria ( Liepiņa *et al*., 2013; Abbas *et al*., 2014; Modilal *et al*., 2015). This has been previously reported where Gram negative bacteria are more resistant to berry extracts and this is explained by the differences in their cell wall structures (Bobinaitė *et al*., 2013). Gram negative bacteria have an outer membrane which acts as a barrier against many external agents. They have hydrophilic channels which only allow entry of hydrophobic substances such as essential oils ( Nikaido, 2003; Burt, 2004; Bobinaitė *et al*., 2013) whereas Gram positive bacteria only have the outer peptidoglycan layer which is more permeable and less effective against antimicrobial compounds (Shen *et al*., 2014). Further, Saleh and Otaibi (2013) demonstrated that Gram negative bacteria are more resistant because they have an outer lipopolysaccharide cell membrane. This could explain the trends that are observed in the current study where Gram negative bacteria were more resistant to the berry extracts while the Gram positive bacteria were more susceptible.

The antimicrobial activity of these berries is indicative of the fact that African nightshade berries can find application in food processing and preservation where they can be used as safe, plant-based preservatives instead of chemical preservatives (Sangija *et al.,* 2021). This could help alleviate some of the health problems and concerns that are associated with chemical preservatives. The results also show that the berries have the potential to be used for medicinal purposes where the antifungal and antibacterial properties are exploited in the pharmacology industry.

**Minimum Inhibitory Concentration (MIC) of Different Varieties of African Nightshade Edible Berries**

Table 3 records the MIC values obtained for the different varieties against the 6 test microbes, tested at the green stage of the berries. MIC values are defined as the lowest concentration of extracts that can inhibit the growth of micro-organisms (Saleh and Otaibi, 2013; Matasyoh *et al*., 2014; Seleshe *et al*., 2017). Most of the extracts exhibited MIC value of 12.50 µg/mL in most bacteria. This was with exception of *E. coli* in which Black N.S extract showed a lower MIC of 6.25 µg/mL. *B. cereus* also had MIC of 6.25 µg/mL in both Black NS and KALRO Agriculture varieties. Fungal species had the highest MIC of all tested microbes, only showing sensitivity at concentrations of 100 µg/mL across all varieties. This further assertion that fungal species are more resistant to the berry extracts as exhibited on Table 2. As such, if the berry extracts were to be used against fungal agents, then higher concentrations would be required.

**Table 3: Minimum Inhibitory Concentration (MIC) Values of Ethanol Extracts from Different Varieties of African Nightshade against 6 Micro-organisms**

| Test Organisms | MIC (µg/mL) | | | |
|---|---|---|---|---|
| | Black NS | Giant NS | JKUAT Impr. | KALRO Agric. |
| *E. coli* | 6.25 | 12.50 | 12.50 | 12.50 |
| *S. typhi* | 12.50 | 12.50 | 12.50 | 12.50 |
| *S. aureus* | 12.50 | 12.50 | 12.50 | 12.50 |
| *B. cereus* | 6.25 | 12.50 | 12.50 | 6.25 |
| *C. albicans* | 100.00 | 100.00 | 100.00 | 100.00 |
| *A. niger* | 100.00 | 100.00 | 100.00 | 100.00 |

Findings from previous works corroborate the findings of the current study. Ethanolic extracts of *Staphylococcus saprophyticus* ATCC 15305 recorded MIC values ranging from 5 – 10 mg/ml against date palm extracts. Seleshe *et al*. (2017) also recorded MIC values ranging from 6.25 to 12.50 µg/mL against *S. aureus, S. pneumoniae, E. coli and K. pneumoniae* using different types of strawberries. However, they concluded that the fungi *A. niger* and *C. albicans* needed concentrations that were above 50 µg/mL. These results underscore the idea that that *S. nigrum* edible berries can be potentiated for use in the pharmacological and food processing industries. This is justified by the fact that the MIC levels were relatable to known MIC levels of the controls; Amphotericin B has a minimum inhibitory concentration (MIC) breakpoint of ≥2 µg/mL (CDC, 2020) while Ampicillin has a breakpoint ranging from ≥ 8 to ≥ 32 µg/mL (FDA, n.d.).

## CONCLUSION

Results from this study indicated that *Solanum nigrum* berries have significant antimicrobial properties. The antimicrobial property of the berries is comparable to other edible fruits especially berries such as strawberries, blue berries, chokeberries among others. The potency of the berries against micro-organisms reduce with ripening with berries at the green and colour break stages having the highest inhibitory effect. The effect also varies with the type of micro-organism used with Gram positive bacteria being more sensitive to the berry extracts compared to the Gram-negative bacteria. The sensitivity of the test microbes could be ranked in the order *S. aureus* > *B. cereus E.* > *Coli* > *S. typhi*. Similarly, it was observed that the berry extracts are more effective against bacterial species as compared to fungal assays. Although all the varieties showed inhibitory quality against the test microbes, it was noted that Black NS and KALRO agriculture varieties were more potent. These antimicrobial properties of the berries could be exploited in the pharmaceutical and food industry.

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