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Original article

In-vitro Anti-bacterial Activity of Ethanolic and Aqueous Leaf Crude Extracts of *Solanum Nigrum* (Black Night Shade) of Bushenyi District - Uganda on Selected Enteric Bacteria

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Abstract

This research was aimed to determine the antibacterial activity of ethanolic and aqueous crude extracts of *Solanum nigrum* leaves against some selected enteric bacteria. Fresh leaves of *S. nigrum* were collected from different garden of Bushenyi district Western Uganda and shade dried. Extraction was done by using standard methods. Phytochemical analyses of both ethanolic and aqueous crude extracts were also done. Antibacterial activities of both aqueous and ethanolic crude extracts were determined against clinical isolates of *Escherichia coli, Klebsiella* sp*, and Shigella sp* and *Salmonella typhimurium* by using agar well diffusion method and compared to the standard antibiotics Ciprofloxacin (5µg/mL) and Cotrimoxazole (25µg/mL). The results of phytochemicals analyses from this study revealed the presence of tannins, alkaloids, and saponins, reducing sugars, terpenoids and steroids from the two extracts. The ethanolic extract was effective only against *E. coli* at concentrations of 1, 0.5 and 0.25g/mL with 20.33±0.33, 15.17±0.17 and 8.33±0.17 mm as mean ± SEM zones of inhibition respectively, while aqueous crude extract was effective against *E. coli* only at concentration of 1g/mL with 9.17±01.7 mm as the mean ± SEM zone of inhibition. The ethanolic crude extract had lower MIC and MBC values of 250 mg/mL and 500 mg/mL respectively compared to the aqueous crude extract with MIC and MBC values of 500 mg/mL and >1000 mg/mL respectively. The results of this study concluded that both ethanolic and aqueous crude extract of *S. nigrum* leaves had activity only against clinical *E.coli*. Ethanolic leaves crude extract of *S. nigrum* was more effective than the aqueous crude leaves extract. This may provide evidences for its usage as herbal remedy against enteric infections caused by *E. coli*.

Keywords: Antibacterial activity, *Solanum nigrum*, Aqueous and ethanolic crude extracts, Enteric-bacteria.

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INTRODUCTION

Gastrointestinal infections (GI) are one of the causes of morbidity and mortality in the developing countries and account for about 50% of all deaths (Stephanie *et al*., 2011; Kotloff *et al.*, 2013, Humphries and Linscott, 2015). The persistencies of these infections may be linked to the poverty of this continent (Stephanie *et al*., 2011; Kotloff *et al*., 2013) and may be facilitated by unsafe water supplies, poor sanitation, and nutritional deficiencies (Humphries and Linscott, 2015). In Uganda, it was estimated that about 89,000 cholera cases and 3,000 deaths occur annually (Ali *et al*., 2015). These infections are majorly caused by microorganisms such as bacteria which include *E.coli, Salmonella* spp, *Shigella* spp and *Klebsiella* spp, *Aeromonas* species, *Campylobacter* species, *Bacillus cereus, Clostridium* spp, *Listeria monocytogenes*, *Plesiomonas shigelloidess, Staphylococcus aureus, Vibrio* and *Vibrio*-Like species, *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* (Humphries and Linscott, 2015). However, previous study has shown that most of diarrhea causing bacteria have developed resistance to the commonly prescribed antibiotics (UNAS, 2015; WHO, 2017). In Uganda for example, antibiotic resistance caused by *Shigella* spp alone seems to be unbearable (UNAS, 2015). Studies conducted between 1997-2007 in different parts of Uganda showed that, *Shigella* isolates were resistant to commonly prescribed antibiotics between 36 and 100% to chloramphenicol, ampicillin and co-trimoxazole, but remained lower – between 0 and < 3% (in three of the four studies) – to quinolones (Legros *et al*.,1998; Mpairwe, 2000; Atwiine, 2007; Kajumbula, 2014). These showed the needs for new, safe and chief antimicrobial agent(s) against resistant organisms.

According to previous studies, 80% of people from low income countries use traditional medicine and utilize plants as drugs for their primary health care needs (Abdala *et al.*, 2012; Anuar *et al*., 2018). These plants contain a wide variety of bioactive compounds that can be used to treat chronics as well as infectious diseases (Duraipandiyanetal., 2006; Madina *et al.*, 2014). In Uganda, the situation is not different as majority of community still rely on traditional remedies for the treatment of many infections (Madina *et al.*, 2014) due to the high cost of conventional medicine which cannot be afforded by common people in the country (Madina *et al.*, 2014). For this reason, there is need for cheap and safe medicine within the country. In western Uganda Bushenyi district, *S. nigrum* (locally called Enshwiiga in lunyankole) is one of the major medicinal herbs used in treatment of enteric infections. But, there is lack of scientific information to validate the antibacterial activity of *S. nigrum* leaves against *S. typhimurium, E. coli*, *Klebseilla* sp and *Shigella* sp that are reported as causative pathogens of most gastrointestinal infections. Although a similar plant has been reported in related studies, locality and ecotypes where the plant is obtained has a significant impact on the concentration of different active phytochemicals (Senjobi *et al.*, 2014). Therefore, this study was aimed to determine the antibacterial activity (minimum inhibitory concentration and minimum bactericidal concentration) of *S. nigrum* leaf aqueous and ethanolic crude extracts collected from different gardens of Bushenyi District, Uganda.

MATERIALS and METHODS

Study design and study area

This was a laboratory experimental study involving the phytochemical analysis of *S. nigrum leaves* extracts and its effect on selected clinical enteric bacteria: *E.coli, S. typhi, Klebsiella sp* and *Shigella sp.* The study was carried out from Microbiology, Pharmacognosy and Biochemistry Laboratories of Kampala International University-Western Campus, Ishaka, Bushenyi District, Uganda. The fresh leaves of *S. nigrum* were collected from different garden of Bushenyi district, Western Uganda (0.4871° S, 30.2051° E).

Collection of plant samples and extraction

The fresh leaves of *S. nigrum* were collected from different gardens in Ishaka-Bushenyi municipality of Bushenyi District, Western Uganda. The leaves were washed thoroughly and then air dried under the shade in pharmacognosy laboratory for 14 days. The dried leaves were crushed using mortar and pestle, then blended by a blender into uniform smaller particles and stored in a container. Crushing and blending enhanced the penetration of the extracting solvents into the plant cells, thus facilitating the release of the active constituents. Hundred grams (100g) of the powdered plant material were extracted in distilled water and ethanol (96%) on a mechanical shaker (Stuart Scientific Orbital Shaker, UK) for 48h. The two extracts were then filtered using a Buchner funnel and filter paper (Whatman No. 1).The resulting extracts were dried in a hot air oven at 40^{o}C for one week. The dried extracts were then weighed and stored in a refrigerator in amber jars. After completion of extraction, the percentage yields of each extract was also calculated using method described by (Pankaj *et al*., 2016)

Phytochemical screening of crude extracts

Phytochemical analysis of the leaves' crude extract was performed according to the method described by (Sofowora, 1993; Pankaj *et al*., 2016). The parameters determined are saponins, tannins, flavonoids, alkaloids, cardiac glycosides, reducing sugars, anthraquinones, polyuronides, steroids, terpenoids and amino acids as follows:

Test for flavonoids: 1.0 mL of 10% lead acetate was added to 1.0 mL of the extract contained in a test-tube. A formation of a yellow precipitate was considered as positive for flavonoids.

Test for tannins: 5.0 g of dried extract was stirred with 10.0 mL of distilled water. The mixture was filtered and ferric chloride reagent was added to the filtrate. A blue-black precipitate was taken as positive for the presence of tannins.

Test for terpenoids: 0.5 mL 0f the dried extracts was evaporated to dryness on a water bath and heated with 3 mL of concentrated sulphuric acid for 10 mins on a water bath. Formation of grey colour was indicating the presence of terpenoids.

Test for cardiac glycosides: 0.5 g of dried extract was dissolved in 2.0 mL of glacial acetic acid containing one drop of ferric chloride solution. The solution was then under laid with 1.0 mL of concentrated H_2SO_4. A brown ring formed at the interface shows the presence of a cardenolides.

Test for saponins: This was screened by shaking 0.5 g of dried extract with water in a test tube, frothing which persist on warming was used as evidence for the presence of saponins.

Test for steroids: 0.5 g of the dried extract was extracted with 2.5 mL of chloroform in a test tube and 1ml of concentrated sulphuric acid added to form a lower layer. A reddish-brown interface was taken as the presence of steroids.

Test for Reducing Sugar

The extract (1 g) was macerated with 20 mL of distilled water and filtered. One millilitre (1 mL) of alkaline copper reagent was added to 1 mL of the filtrates. The mixture was boiled for 5 min and allowed to cool. After cooling, the mixture was added with 1 mL of phosphomolybdic acid reagent and 2 mL of distilled water and the absorbance read at 420 nm.

Test for Amino acid (Ninhydrin test)

The extract (2.0 g) was dissolved in 10 mL of acetone or ethanol. Few drops of 2% Ninhydrin solution was added to the mixture. The mixture was kept in water bath for 5 min. Blue or violet color development was taken as presence of Amino acid.

Test for Anthraquinone

One gram (1 g) of the ground leaf was placed in a dry test tube and supplemented with 20 mL of chloroform. This was heated in steam bath for 5 min. The extract was filtered immediately and allowed to cool. An equal volume of 10% ammonia solution was added to the filtrate. The mixture was shaken and the upper aqueous layer was observed for bright pink colouration which was used as indicative of the presence of Anthraquinones.

Preparation of test organism inoculums

Escherichia coli, Salmonella typhi, Klebsiella spp. and Shigella spp. were obtained as stock clinical cultures from Microbiology Laboratory of Kampala International University Western Campus Ishaka Bushenyi. The test organisms were sub cultured in nutrient agar and incubated at 37℃ for 24 hours to check the viability of the organisms. The concentration of the test organisms were adjusted to turbidity standard of 0.5 McFarland standard (1-2.0x10^5cfu/mL) as described by (Senjobi *et al*., 2017).

Preparation of extract concentrations

Four different concentrations of each extract were prepared in mg/mL (1000, 500, 250 and 125 mg/mL) using 10% Dimethyl Sulphur Oxide (10 % DMSO) as diluent (Mishra *et al*., 2017).

Antibacterial susceptibility test

An antibacterial susceptibility test was done according to the guideline set by the Clinical and Laboratory Standards Institute (CLIS, 2014, 2018). Standardized test organism (0.5 McFarland standards) was inoculated on freshly prepared plates of Mueller Hinton agar (HiMedia Laboratories Pvt Ltd, Mumbai, India, M173) using sterile cotton swab. Wells were made using sterile cork borer (6mm). Wells were filled with 50 µL of each concentration (1000, 500, 250 and 125mg/mL) of crude extract. Ciprofloxacin 5 µg/mL and Cotrimoxazole 25 µg/mL were used as positive controls while 10% DMSO was used as negative control. This was left in inoculating chamber for 30 min to allowed extract to diffuse, after which plates were incubated at 37ºC for 24h. After incubation period, the diameters of zones of inhibition were measured in millimetres (mm) with ruler (Vandepette, 1991) and results were interpreted according to (CLIS, 2014, 2018) guideline. The experiment was performed in triplicates.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration and the minimum bactericidal concentration of *S. nigrum* ethanol leaves crude extract was determined for susceptible test bacteria using a two-fold serial broth dilution method in test tubes according to Gurnani *et al*. (2016); Mathur, (2013). Zero point five millilitre (0.5mL) of initial concentration (4000 mg/mL) of ethanolic crude extract of *S. nigrum* leaves was serially diluted in test tubes containing 0.5 mL of freshly sterilized Brain Heart Infusion (BHI) to obtained different concentrations ranging from 2000, 1000, 500, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91 and 1.95 mg/mL. Test organism suspension was prepared in 0.85% normal saline and its turbidity was adjusted to standard 0.5 McFarland equivalent to 1.5×10^8 cfu/mL. This was further diluted by transferring 0.1 mL from the standardised organism suspension into a tube containing 9.9 mL 0.85% normal saline to give a final cell density of $1.0x10^6$ cfu/mL which was used in the experiment. The standardized organism suspension 0.5 ml ($1.0x10^6$ cfu/mL) was added in each of the tube containing the serially diluted crude extract. This was mixed to homogeneity to give a final inoculum of $5x10^5$ cfu/mL (Arendrup, 2012; Perumal *et al*., 2012). Two positive control tubes were used: one test tube with broth and test organism while the other one contained the broth only aimed at checking ability of used broth media to support test bacteria growth (bacteria viability) and sterility of broth respectively. The third control tube contained broth and crude extract aimed at ascertaining for any prior microbial contamination of the extract. The inoculated tubes were incubated at 37℃ for 24 hours. After the incubation period, blanks for each tube concentration (extract and BHI only) were prepared and this was followed by examination of inoculated tubes for visible turbidity and optical density reading at 600nm using a Beckman DU-70 UV-Vis Spectrophotometer. The optical density of each inoculated tube was compared with its respective blank tube. MIC of the crude extract was considered as the lowest concentration that had optical density equivalent to its respective blank tube, thus had no visible bacterial growth. The test experiment assay was performed in triplicates (Bussmann *et al*., 2010).

Determination of Minimum Bactericidal Concentration (MBC)

A loop full from each of the tubes with no visible growth after 24 hours were sub-cultured on freshly prepared Mueller–Hinton agar by the streak plate method and were incubated at 37ºC for 24 hours. The plates were examined for any colony growth and then observed for growth. The least concentration from at which no colony growth was observed was considered as the minimum bactericidal concentration (Obiagwu *et al.*, 2011).

Data analysis

Data was entered into Microsoft Excel and then exported to SPSS- version 16 for analysis to compute descriptive statistics of mean and standard error of mean (SEM) inhibition zone diameter. Data was also analysed with Graph Pad prism 6 to perform One-way Analysis of Variance (ANOVA) using a Tukey's multiple comparison test to compare between antibacterial activities of different extract concentrations against each test bacteria versus controls. Two-way ANOVA using Sidak's multiple comparisons test was used to determine if there were significant differences in the antibacterial activities between the aqueous and ethanolic extracts at varying concentration against *E. coli*. Statistical significance was considered at p- value ≤ 0.05 was considered significant. The activity indices were of the crude extracts was calculated against both standard control antibiotics used. The experimental results of antibacterial susceptibility were expressed as means ± SEM and presented in form of tables and graphs.

RESULTS and DISCUSSION

The results of percentage yield of aqueous and ethanolic leaves crude extracts of *S. nigrum* is shown in Table 1. The result showed that both aqueous and ethanol leaves crude extract had 8.91 and 8.28% percentage yield extract respectively. It was observed that the percentage yield of the aqueous leaves crude extract was slightly higher than that of ethanolic crude extract; this may be due to the high solubility of the plant's phytochemicals and other components in water than ethanol. This showed varying extractive potentials for the solvents used as the aqueous solvent exhibited a larger extractive ability thus increasing the amount extracted from the plant.

Table 1. Percentage yield of *S. nigrum* leaves aqueous and ethanolic crude extracts.

Extracts	Weight of powder residue (g)	Weight of extract (g)	Percentage yield (w/w)
Aqueous	100	9.18	8.91
Ethanol	100	8.26	8.26

The results of phytochemical analysis in Table 2 showed that, both aqueous and ethanolic leaves crude extract had alkaloids, reducing sugars, saponins, tannins and terpenoids. In addition ethanolic leaves crude extract contained steroids and flavonoids. The absence of steroids and flavonoids from

aqueous leaves crude extract could be due to the inability of flavonoids and steroids to dissolve in aqueous. This study was in line with previous study by (Pankaj *et al*., 2016) from India who reported the presence of saponins, tannins, alkaloids, terpenoids, flavonoids, glycosides, and steroids as well as the presence of proteins from upper part of *S. nigrum* (leaves, stems and fruits). Rajathi *et al*., 2015 also reported the presence of alkaloids, flavonoids, steroids, tannins from aqueous and ethanolic leaves crude extract of *S. nigrum*. However, saponins and terpenoids were absent while phlobatannins were detected in the ethanolic crude extract of the leaves. George *et al*. (2017) from Kenya reported the presence of alkaloids, tannins, saponins, terpenoids, flavonoids, cardiac glycosides and phenols as well as absence of steroids. This confirmed the statement by Senjobi *et al*., (2017) who reported that plant from different geographical area across the world may have different combinations and concentrations of the active substances.

Table 2. Phytochemical screening of the aqueous and ethanolic leaves crude extracts of *S. nigrum*

Constituents	Crude extracts	
	Ethanol extract	**Aqueous extract**
Alkaloids (Wegner's test)	+	+
Reducing sugars (Benedict test)	+	+
Saponins (Frothing test)	+	+
Flavonoids (Shinoda's test)	+	-
Tannins	+	+
Test for steroids	+	-
Terpenoids (Salkowski test)	+	+
Test for cardiac glycoside	-	-
Amino acids (Ninhydrin test)	-	-
Test for Anthraquinones	-	-

Key: (+) = Present, (-) = Absent.

The results of antibacterial activity of aqueous leaves crude extract of *S. nigrum* against the test bacteria are presented in Table 3. The results showed that aqueous leaves crude extract had activity only on *E. coli* at concentration of 1000 mg/mL with mean ± SEM zone of inhibition of 9.17 ± 0.17mm which was statistically different (p= < 0.0001) from negative control (10% DMSO), 500, 250 and 125 mg/mL which showed no activity. However, the activity exhibited at concentration of 1000 mg/mL was significantly lower than that showed by positive controls; Ciprofloxacin, 5µg/mL and Cotrimoxazole, 25µg/mL (Table 5, Figure 1). Table 4 shows that *S. nigrum* ethanolic leaves crude extract exhibited antibacterial activity against only *E. coli*. The ethanolic leaves crude extract showed activity against *E. coli* at concentrations of 1000, 500 and 250 mg/mL with mean ± SEM zone of inhibition of 20.33 ± 0.33 mm, 15.17 ± 0.17 and 8.33 ± 0.17 mm respectively (Table 4). Using Tukey's multiple comparisons test, the activity of ethanolic leaves crude extract at concentrations of 1000, 500 and 250 mg/mL against *E. coli* was significantly different (p= < 0.0001) from concentration at 125 mg/mL and negative control (10% DMSO) which showed no activity. Furthermore, the ethanolic extract concentration at 1000

mg/mL showed significantly (p= < 0.0001) higher activity against *E. coli* as compared to 500, 250 and 125 mg/mL. The ethanolic extract at concentration of 500 mg/mL showed significantly (p= < 0.0001) higher activity against *E. coli* compared to that showed at concentration of 250 mg/mL (Table 5, Figure 1). However, the extract concentrations at 1000, 500 and 250 mg/mL showed statistically (p= < 0.0001) lower antibacterial activity against *E. coli* as compared to the positive controls; Ciprofloxacin, 5µg/mL and Cotrimoxazole, 25µg/mL (Table 5, Figure 1). Furthermore, *Salmonella typhimurium* and *Klebsiella* spp. were not susceptible to antibiotic standards (Cotrimoxazole, 25µg/mL) as compared to *E. coli* and *Shigella* spp. which were susceptible to all standard antibiotics used in the current study. No activity was observed from negative control (10% DMSO). The ability of the *S. nigrum* leaves aqueous and ethanolic crude extracts to show activity against *E. coli* could be due to the presence of bioactive compounds (alkaloids, reducing sugars, saponins, tannins, terpenoids, steroids and flavonoids) in the crude leaves extracts (Table 2). These bioactive compounds were reported to have antimicrobial activity (Rajathi *et al*., 2015). Rajathi *et al.* added that different researchers (Mandal *et al*., 2005; Shahiladevi and Jegadeesan, 2017; George *et al*., 2017) reported the presence of these compounds from different plants sources possessing anti-viral, anti-bacterial, anti-fungal, anti-helminthic and anti-inflammatory properties. For example, tannins were reported to have antimicrobial and antioxidant activities as well as they serve as cytotoxic and antineoplastic agents (De-Lucca *et al*., 2005; Mohanta *et al*., 2011; Rajathi *et al*., 2015). Two-way ANOVA using Sidak's multiple comparisons tests was done to compare antibacterial activity between aqueous and ethanolic crude extracts against *E. coli* (Table 6, figure 2). The results showed that ethanolic crude extracts (1000-250 mg/mL) had significantly (p= < 0.0001) higher antibacterial activity compared to the same concentrations of aqueous extracts. However, both extracts had no activity against *E. coli* at concentration of 125 mg/mL. The variability of antibacterial activity of the two different leaves crude extracts of *S. nigrum* (aqueous and ethanolic) against *E. coli* could be due to the differences in bioactive compounds present in these leaves crude extracts. This is because ethanol used as a solvent during the extraction process is known to have better extraction potential for most of the polar phytochemicals as compared to water. This was in line with Pankaj *et al.* (2016); Shahiladevi and Jegadeesan (2017) who reported higher activity of ethanolic crude extract against *E. coli*, although the former study used whole plant crude extract. In addition, Pankaj *et al.* (2016) reported activity of both ethanolic and aqueous leaves crude extracts against *S. typhimurium* which was contrary to our finding. The inability of ethanolic and aqueous leaves crude extracts used in this study to show activity against *Salmonella typhi*, *Klebsiella* sp and *Shigella* sp may be due to the differences in the genetic makeup of these organisms or these organisms may expose to similar bioactive compounds before, therefore developing resistance against current tested bioactive compounds. This was not surprising because the history of these organisms showed that, they were isolated from clinical samples of patients from community which reportedly claimed to be using *S. nigrum* concoction in the treatment of gastrointestinal infections.

The results of this study showed that, standard antibiotics are more active than the *S. nigrum* leaves aqueous and ethanolic crude extracts against *E. coli* (Table 5, figure 1). This could be attributed by the extracts being in the crude form containing various compounds that may have antagonistic activity against other bioactive phytochemicals resulting to the lower antibacterial activity against tested organism as compared to the standard positive control antibiotics used (Iqbal and Farrukh, 2007).

Table 3. Inhibition zone diameters of aqueous leaves crude extracts of *S. nigrum* against test bacteria

	Mean inhibition zone diameter ± SEM in mm			
Concentrations of aqueous extract and Controls	***Salmonella typhimurium***	***E. coli***	***Klebseilla* sp**	***Shigella* sp**
Ciprofloxacin (5µg/mL)	29.50 ± 0.29	37.33 ± 0.33	36.83 ± 0.17	26.67 ± 4.84
Cotrimoxazole (25µg/mL)	0	38.33 ± 0.17	0	11.50 ± 5.75
DMSO (10%)	0	0	0	0
1000 mg/mL	0	9.17 ± 0.17	0	0
500 mg/mL	0	0	0	0
250 mg/mL	0	0	0	0
125 mg/mL	0	0	0	0

Key: mm = millimetre SEM= Standard Error of Mean

Table 4. Inhibition zone diameters of ethanolic leaves crude extracts of *S. nigrum* against test bacteria

	Mean inhibition zone diameter ± SEM in mm			
Concentrations of ethanolic extract and Controls	***Salmonella typhimurium***	***E. coli***	***Klebseilla* sp**	***Shigella* sp**
Ciprofloxacin (5µg/mL)	29.50 ± 0.29	37.33 ± 0.33	36.83 ± 0.17	26.67 ± 4.84
Cotrimoxazole (25µg/mL)	0	38.33 ± 0.17	0	11.50 ± 5.75
DMSO (10%)	0	0	0	0
1000 mg/mL	0	20.33 ± 0.33	0	0
500 mg/mL	0	15.17 ± 0.17	0	0
250 mg/mL	0	8.33 ± 0.17	0	0
125 mg/Ml	0	0	0	0

Table 5. Multiple comparisons between different concentrations of *S. nigrum* leaves aqueous and ethanolic crude extracts versus controls against *E. coli*

Tukey's multiple comparisons test	**Adjusted *P-values***	
	Aqueous extract	**Ethanolic extract**
Ciprofloxacin (5µg/mL) vs. Cotrimoxazole (25µg/mL)	0.0099**	0.0099**
Ciprofloxacin (5µg/mL) vs. DMSO (10%)	< 0.0001****	< 0.0001****
Ciprofloxacin (5µg/mL) vs. 1000 (mg/mL)	< 0.0001****	< 0.0001****
Ciprofloxacin (5µg/mL) vs. 500 (mg/mL)	< 0.0001****	< 0.0001****
Ciprofloxacin (5µg/mL) vs. 250 (mg/mL)	< 0.0001****	< 0.0001****
Ciprofloxacin (5µg/mL) vs. 125 (mg/mL)	< 0.0001****	< 0.0001****
Cotrimoxazole (25µg/mL) vs. DMSO (10%)	< 0.0001****	< 0.0001****
Cotrimoxazole (25µg/mL) vs. 1000 (mg/mL)	< 0.0001****	< 0.0001****
Cotrimoxazole (25µg/mL) vs. 500 (mg/mL)	< 0.0001****	< 0.0001****
Cotrimoxazole (25µg/mL) vs. 250 (mg/mL)	< 0.0001****	< 0.0001****
Cotrimoxazole (25µg/mL) vs. 125 (mg/mL)	< 0.0001****	< 0.0001****
DMSO (10%) vs. 1000 (mg/mL)	< 0.0001****	< 0.0001****
DMSO (10%) vs. 500 (mg/mL)	> 0.9999	< 0.0001****
DMSO (10%) vs. 250 (mg/mL)	> 0.9999	< 0.0001****
DMSO (10%) vs. 125 (mg/mL)	> 0.9999	> 0.9999
1000 (mg/mL) vs. 500 (mg/mL)	< 0.0001****	< 0.0001****
1000 (mg/mL) vs. 250 (mg/mL)	< 0.0001****	< 0.0001****
1000 (mg/mL) vs. 125 (mg/mL)	< 0.0001****	< 0.0001****
500 (mg/mL) vs. 250 (mg/mL)	> 0.9999	< 0.0001****
500 (mg/mL) vs. 125 (mg/mL)	> 0.9999	< 0.0001****
250 (mg/mL) vs. 125 (mg/mL)	> 0.9999	< 0.0001****

P-values obtained from one-way ANOVA, " ** " and " **** " shows statistical significance at 95% level of confidence.

Table 6. Sidak's multiple comparisons between antibacterial activity of aqueous and ethanolic crude extracts of *S. nigrum* leaves against *E. coli*

Multiple comparisons test: Aqueous Vs Ethanolic	***Adjusted P Value***
Ciprofloxacin (5µg/mL)	*> 0.9999*
Cotrimoxazole (25µg/mL)	*> 0.9999*
DMSO (10%)	*> 0.9999*
1000 (mg/mL)	*< 0.0001***
500 (mg/mL)	*< 0.0001***
250 (mg/mL)	*< 0.0001***
125 (mg/mL)	*> 0.9999*

P-values obtained from two-way ANOVA, " **** " shows statistical significance at 95% level of confidence.

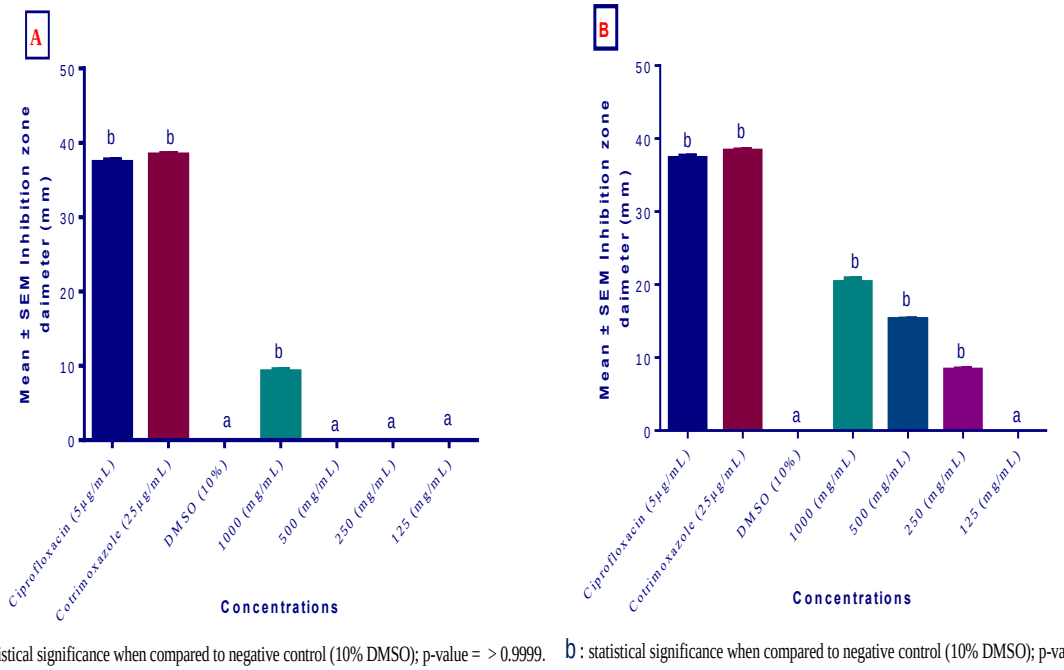

Figure 1. Tukey's multiple comparison of antibacterial activity of *S. nigrum* leaves crude extracts. One-way Analysis of Variance (ANOVA). **A**- Aqueous leaves crude extract, **B**- Ethanolic leaves crude extract

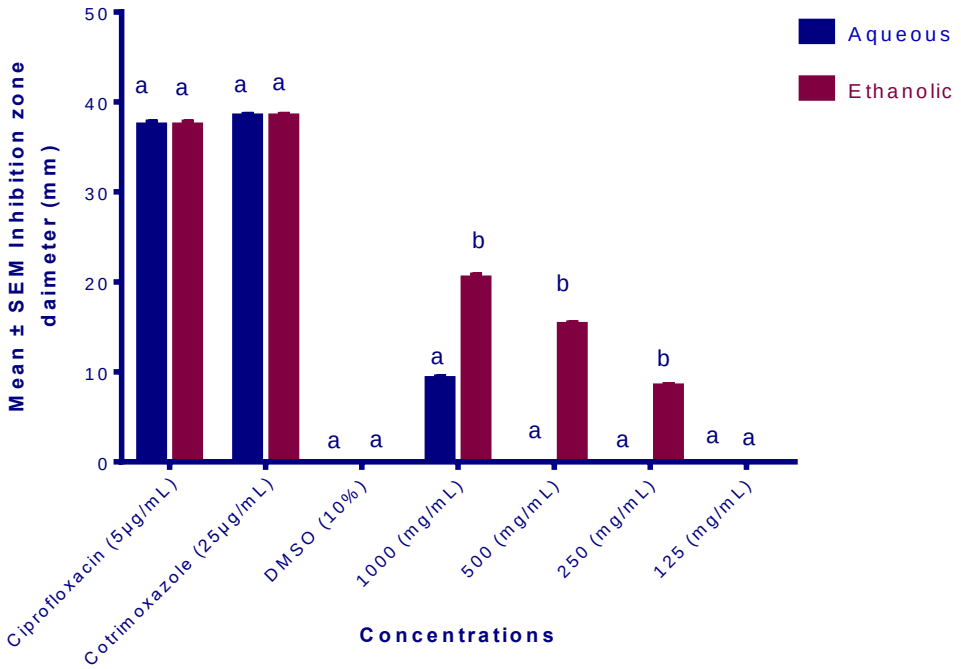

Figure 2. Sidak's multiple comparisons between antibacterial activity of aqueous and ethanolic crude extracts of *S. nigrum* leaves against *E. coli*. Two-way Analysis of Variance (ANOVA).

This study determined the minimum inhibitory concentration and minimum bactericidal concentration of the ethanolic and aqueous leaves crude extract of *S. nigrum* against *E. coli* (Table 7). The results showed that, ethanolic crude extract had a lower MIC of 250mg/mL compared to the aqueous crude extract 500 mg/mL. The MBC of ethanolic leaves crude extract of *S. nigrum* against *E. coli* was found to be 500 mg/mL while aqueous crude leave extract was found to have more than 1000 mg/mL.

Table 7. Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of the ethanolic leaves crude extract of *Solanum nigrum* against *E .coli.*

Test bacteria	***Escherichia coli***	
	Ethanolic crude extract	**Aqueous extract crude**
MIC (mg/mL)	250	500
MBC (mg/mL)	500	>1000

Conclusion

This study revealed the presence of phytochemicals: tannins, alkaloids, and saponins, reducing sugars, terpenoids and steroids from *S. nigrum* leaves aqueous and ethanolic crude extracts. It was also showed that the *S. nigrum* leaves aqueous and ethanolic crude extracts were had activity to only *E.coli* and lacked activity against *Salmonella typhi, Klebseilla sp* and *Shigella sp.* The study validated the use of concoctions prepared from *S. nigrum* leaves in the treatment of infections commonly caused by *E. coli*

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REFERENCES

Abdala S, Martín-Herrera D., Benjumea D., Gutiérrez, S.D. (2012). Diuretic activity of some Smilax canariensis fractions. J Ethnopharmacol. 140: 277-81.

Ali M., Nelson, A.R., Lopez A.L., Sack D.A. (2015) Updated Global Burden of Cholera in Endemic Countries. PLoS Negl Trop Dis. 9(6), e0003832. doi:10.1371/journal.pntd.0003832

Anuar, S.G., Saudy S. P.P., María Mirian E.C., María E. M.-C. (2018). Diuretic activity of aqueous extract and smoothie preparation of Verbesina crocata in rat. Bangladesh J Pharmacol. 13: 236-240

Atwiine, A. K. (2007). Determination of Circulating *Shigella* Serotypes and their antimicrobial Susceptibility Patterns in selected Districts of Uganda. (Bachelor of Biomedical Laboratory Technology), Makerere University, Kampala.

Bussmann, R.W., Malca-García, G., Glenn ,A., Sharon, D., Chait, G., Díaz ,D., Pourmand, K., Jonat, B., Somogy, S., Guardado,G., Aguirre,C., Chan, R., Meyer, K., Kuhlman, A., Townesmith, A., Effio-Carbaja, J., Frías-Fernandez, F., and Benito, M. (2010). Minimum inhibitory concentrations of

medicinal plants used in Northern Peru as antibacterial remedies. J. Ethnopharmacol. 132(1), 101–108.

Clinical and laboratory standards institute (CLSI) (2018). Document M100. Performance Standards for Antimicrobial Susceptibility Testing, 28th Edition A CLSI supplement for global application.

CLSI. 2014. Performance standards for antimicrobial susceptibility testing; twenty-fourth informational supplement, M100 S24. Clinical and Laboratory Standards Institute, Wayne, PA.

De-Lucca, A., Cleveland, Ts., Rajasekara, K., Boue, S., Brown,R., 2005. Fungal properties of CAY-1, a plant saponin, for emerging fungal pathogens. *45th Inter Science Conference in Antimicrobial Agents and Chemotherapy Abstract*, 180.

Duraipandiyan, V., Ayyanar, M.,I gnacimuthu, S., 2006. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC Complementary and Alternative Medicine. 6, 35–41.

Arendrup, M. C., Cuenca-Estrella, M., Lass-Flörl, C., Hope, W., & EUCAST-AFST. (2012). EUCAST technical note on the EUCAST definitive document EDef 7.2: method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for yeasts EDef 7.2 (EUCAST-AFST). Clinical Microbiology and Infection. 18(7), E246-E247.

George, T., Opande, D. M., Musyimi, K. L., Cherono, B. K., Jiveri, D. K. B. (2017). Antimicrobial activities of crude leaf extract of *Solanum nigrum* on *Fusarium oxysporum* and *Pseudomonas syringae* cultures in Maseno (Kenya). Journal of Asian Scientific Research. Vol. 7, No. 7, 271-278.

Gurnani, N., Gupta, M., Shrivastava, R., Mehta, D., Mehta, B. K. (2016). Effect of extraction methods on yield, phytochemical constituents, antibacterial and antifungal activity of Capsicum frutescens L.

Humphries, R.M., Linscott, A.J. (2015). Laboratory diagnosis of bacterial gastroenteritis. Clin Microbiol Re. 28:3–31. doi:10.1128/CMR.00073-14.

Iqbal A., Farrukh A. (2007). In vitro efficacy of bioactive extracts of 15 medicinal plants against ESbL-producing multidrug-resistant enteric bacteria. Microbiological Research 162 (2007) 264—275

Kajumbula, H. (2014). Antimicrobial Resistance (T. D. o. M. Microbiology, Trans.): Makerere University College of Health Science.

Kotloff, K.L., Nataro, J.P., Blackwelder, W.C., Nasrin, D., Farag, T.H, Panchalingam S, *et al*. (2013). Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. Lancet. 20; 382 (9888):209–22.

Legros, D., Ochola, D., Lwanga, N., Guma, G. (1998). Antibiotic sensitivity of endemic *Shigella* in Mbarara, Uganda. *East Afr Med.* 75(3); 160-161.

Madina, M. A., Godwin, A., Robert B., Maud, K.M., Yahaya S., Esezah, K. K, Bernard T. K. (2014).Medicinal plants used in malaria treatment by Prometra herbalists in Uganda. *Journal of Ethnopharmacology*. 155(2014)580–588.

Mandal, P., Babu, S. and Mandal, N., 2005. Antimicrobial activity of Saponins from *Acacia auriculiformis*. *Fitoterapia*, 76(5): 462-565.

Mathur, R. (2013). Phytochemical and antimicrobial evaluation of plant extracts of Enicostemma hyssopifolium. Journal of Pharmacognosy and Phytochemistry, 2(4), 30-36.

Mishra, M. P., Rath, S., Swain, S. S., Ghosh, G., Das, D., & Padhy, R. N. (2017). In vitro antibacterial activity of crude extracts of 9 selected medicinal plants against UTI causing MDR bacteria. Journal of King Saud University-Science, 29(1), 84-95.

Mohanta, T., Patra, J., Rath, S., Pal, D. and Thatoi, H., 2011. Evaluation of antimicrobial activity and phytochemical screening of oils and nuts of *Semicarpus anacardium*. *Lif. Sci. Res. Essay*, 2(11): 486-490.

Mpairwe, Y. (2000). Resistance Pattern of *Shigella* Isolates at Naguru Medical Laboratory, Kampala (1995 and 2000) Naguru Medical Laboratory, Kampala.

Obiagwu, I. N., Okechalu, O.B., Njoku, M.O. (2011). Studies on Antibacterial Effect of The Leaves Of *Phyllanthus niruri* on Some Enteric Pathogens. *Nig J. Biotech*. 23; 22- 27 I.

Pankaj Kumar, Jitendra Kumar, Raghuveer Kumar and R. C. Dubey (2016). Studies on phytochemical constituents and antimicrobial activities of leaves, fruits and stems of *Solanum nigrum* L. *Asian Journal of Plant Science and Research*, 6(4):57-68

Perumal, S., Pillai, S., Cai, L. W., Mahmud, R., & Ramanathan, S. (2012). Determination of minimum inhibitory concentration of Euphorbia hirta (L.) extracts by tetrazolium microplate assay. Journal of Natural Products, 5(2).

Rajathi M. D Modilal, R. Anandan, R. Sindhu and M.N. Logeshwari (2015). Screening of solanum nigrum for its phytochemical and antimicrobial activity against respiratory tract pathogens. International Journal of Pure and Applied Zoology Volume 3, Issue 3, pp: 210-215

Senjobi, C.T., Ettu, A. O. and Otujo, C.O. (2017). Antibacterial and antifungal activities of leaf extracts of *Phyllanthus amaru* Schum and Thonn. Journal of Pharmacognosy and Phytotherapy. Vol. 9(1), pp. 6-10

Stephanie, M., Fletcher, Damien Stark, and John Ellis (2011). Prevalence of gastrointestinal pathogens in Sub-Saharan Africa: systematic review and meta-analysis. J Public Health Africav. 2(2)

Sofowora E.A., (1993). Medicinal Plants and Traditional Medicine in Africa.John Wiley and Sons Inc.New York, pp256-257.

Shahiladevi S. and Jegadeesan M. (2017). Antimicrobial Activity of Black Fruit Variant of *Solanum nigrum* L. International Journal of Current Microbiology and Applied Sciences ISSN: 2319-7706 Volume 6 Number 6 pp. 2706-2713

Uganda National Academy of Sciences (UNAS) "Antibiotic Resistance in Uganda: Situation Analysis and Recommendations, ISBN: 978-9970-424-10-8. A4 Lincoln House Makerere University P.O. Box 23911, Kampala, Uganda, 2015.https://cddep.org/wp-content/uploads/2017/08/garp-uganda_sa.pdf

WHO (2017). WHO guidelines on good herbal processing practices (GHPP) for herbal medicines. Available at http://www.who.int/medicines/areas/quality_safety/quality_assurance/V-2ndGlobalReview-RevisedDraft-WHO-GHPP-March2017.pdf. Accessed 9/8/2018