

Effects of Ethanolic Extracts of *Solanum aethiopicum* Leaf on Haematological parameters of Wistar Rats Induced Kidney Infections

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Abstract

This evaluated the effects of leaf extracts of *Solanum aethiopicum* on haematological parameters of wistar rats induced bacteria isolated from urinary tract infections in a view to developing a herbal drug that is capable of replacing resistant antibiotics at a very low cost and available always. The phytochemical analyses of crude extracts were evaluated spectrophotometrically; isolates were obtained and identified culturally, morphologically and biochemically. Isolates were inoculated into rats via peritoneal method and treatment was done orally. Both antibiotic susceptibility test of the isolates to commercial drugs and crude extracts were studied via disc diffusion and agar well diffusion methods respectively. The effect of the leaf extract was evaluated by treating rats (WBC count 4.5×10^3) with sub-MIC of *Solanum aethiopicum* extracts infected with isolates and appearance of disease septum with 11.3×10^3 WBC count after 5 days from infection. The photochemical analysis revealed the presence of alkaloids; phenolics, tannins, saponins, flavonoids, steroids and glycosides. The ethanolic extract exhibited more activity than the aqueous extract against *S. aureus* most followed by *Streptococcus sp.*, *E. coli* and *Klesiella sp.* was the least. After treating the infected mice with the plant extract and after 6 days from infection, the WBC count reduced to $5.1 \times 10^3 \mu l$ which is normal range and the rats were healthy with good physiological behaviour. The infected mice and untreated with extracts, the WBC and other immunological parameters remain high even after 16 days from infection. The study showed that *Solanum aethiopicum* possessed antibacterial properties and should serve as alternative therapy for ameliorating urinary tract infections.

Keywords: Leaf; Nkpuruofe; Afufa; Haematology Renal Function; *Solanum aethiopicum*

Introduction

Urinary tract infections are treated with antibiotics and untreated case could cause permanent damage of kidneys (pyelonephritis); however, pathogens of cystitis are fast becoming resistant to some antibiotics if not all. This resistance has increased the interest of medical researchers on herbal remedies that can serve as alternatives to conventional antibiotics; in line with this quest, this work x-rayed the effects of both ethanol and aqueous extracts of *Solanum aethiopicum* leaf on haematological parameters with a view to developing an effective antimicrobial agent for the treatment of urinary tract infections. *Solanum aethiopicum* commonly known as “Anyara” in Southeastern Nigeria also called African eggplant, Ethiopian eggplant or scarlet eggplant is a vegetable crop belonging to the family *Solanaceae*. The genus *solanium* includes both the edible and non-edible species (Prohens, *et al.* 2015). They are native to sub Saharan Africa and are essentially tropical in origin. *S. aethiopicum* is of high edible quality [1-5].

The physiological efficacy of the plant is losen and temporarily such nervous actions as is reflected to distant organs of the body from some central organs which is the actual seat of trouble (Hoyto, 2011). In this way, the spasms of asthma and other convulsions with similar symptoms are allowed to be treated with large doses of the plant. Young children have been attacked with bronchoconstriction after inhaling freely of the allergens. In Indian work of domestic remedies carried out in 1999, eggplant was considered of great curative power in diseases (Vasudev, 2009) [6-9].

Aside from their nutritional roles in complimenting staple foods to form balanced diets, they also influence biochemical parameters in the body (Sofawara, 2013). Such influence when positive helps the body to fight many disease conditions (Elujoba, *et al.*, 2015). Report has shown that *S. aethiopicum* possesses ulcer protecting properties against experimentally induced ulcers in rats (Ezeugwu *et al.*, 2014), has a reducing effect on lipid profile of Wister albino induced lipidemia (Nwodo *et al.*, 2013), and showed weight reducing effect as well as hypolipidemic properties (Ossamul, *et al.*, 2014). They are used to treat severe pain resulting in periodic spasm in an abdominal organ and blood pressure (Grubben, 2014). Other reports on the pharmacological activity of the plant show that it has purgative (Saba *et al.*, 2013), sedative and anti-diabetic effects (Ezeugwu, *et al.*, 2014). The leafy part of *Solanum aethiopicum* is also applied to areas of skin disease, infections, and sores (Oliver-Beaver, 2015).

Considering the rate at which the fruit of this plant is being consumed within Nigeria, there is the need to look at the effect of the leaf, on some biochemical paramrtrers [10-16].

Report had it also that *S. aethiopicum* possesses ulcer protecting properties against experimentally induced ulcer in rats (Chioma, *et al.*, 2012). They are used to treat colic, severe pain resulting in periodic spasm in an abdominal organ and blood pressure (Grubben *et al.*, 2014). Other reports on the pharmacological activity of the plant show that it has purgative (Saba, *et al.* 2013), sedative and anti-diabetic effects (Ezeugwu, *et al.*, 2014) [17-19].

In indigenous medicine, *S. aethiopicum* has a wide range of utilization from weight reduction to treatment of several ailments including asthma, allergic disease, swollen joint pains, gastro-esophageal reflux disease, constipation, and dyspepsia. Scientific studies have supported the traditional use of this plant in treating inflammation, asthma, glaucoma, diabetes and excessive weight gain. The fruit is easily eaten as a snack and it has been reported to be high in phytochemicals like saponins, flavonoids, tannins and ascorbic acid (Nwodo, *et al.* 2013) [20-23]. Studies have shown that dyslipidemia-associated non-communicable diseases like diabetes and obesity are on the increase in the developing world and a continuous study is required to identify indigenous plant materials that can mitigate against, or at least useful in the management of, dyslipidemia (Dalal, 2011, Nwodo *et al.*, 2013) [24-26].

In developing countries, remedies from plants are readily used in the treatment of various kinds of diseases. Different medicinal plants possess diverse therapeutic potential as no single plant has all the medicinal properties (Ghasi, *et al.*, 2011). Many of the medicinal potentials of plants used in folkloric medicine have been subjected to scientific investigation and this has warranted their widespread use as an alternative or complement to orthodox medicines. However, the medicinal potential of African flora is yet to be fully explored. Some plants of the African vegetation are yet to be discovered for their medicinal properties. This study was aimed at determining the qualitative and quantitative phytochemical composition of ethanol extract of *Solanum aethiopicum* leaf as well as evaluating its effect on the hematology indices of Wistar albino rats [27-30].

Materials and Methods

Sample collection and classification

This was carried out in Awka, Nsukka and Lagos. Samples (urine) were collected from University Teaching Hospital Itiku Ozalla (UNTH) Enugu, Nnamdi Azikiwe University Teaching Hospital Nnewi and Chukwuemeka Odumegwu Ojukwu University Teaching Hospital Amaku- Awka.

Collection of Urine sample was as follows:

In this study, a total of 350 urine samples were collected between June and September 2019 according to Slovins (2013) as shown below from 3 different teaching hospitals which include, University of Nigeria Teaching Hospital Itiku Ozalla (UNTH) Enugu, Nnamdi Azikiwe University Teaching Hospital Nnewi and Chukwuemeka Odumegwu Ojukwu University Teaching Hospital Amaku- Awka from patients suffering from urinary tract infections (UTIs) and attending treatment. They were classified according to name, gender and age of patients. The patients were given sterile bottles and instructed on how to collect clean, mid-stream, voided urine samples. Patients unable to collect their own were aided by medical experts. (Monica Cheesbrough *et al.*, 2015) [31-35].

Collection of leaf samples

Collection, authentication and processing of plant materials

Collection, authentication and processing of plant material: fresh leaves of *Solanum aethiopicum* were collected from the Agric farm of Faculty of Agricultural Sciences of the University of Nigeria, Nsukka. Plant material was identified by Dr. Ugwuozor, a taxonomist of Botany Department of University of Nigeria, Nsukka. Taxonomic identity of plants were achieved (No: 1120) by deposited voucher specimen and use of documented literature from Dalziel (2016) in the herbarium unit of Department of Botany, University of Nigeria, Nsukka.

Isolation and Identification of Test Organism

All *E.coli* recovered from 350 urine samples which collected from patients with urinary tract infections and attending treatment from June -November, 2020 and identified via cultural

properties (LAB/ United Kingdom), microscopical examination followed by biochemical tests (Catalase, Methyl-Red, Oxidase, Indole, Voges-Proskauer Test, Citrate Utilization, Sugar Fermentation and CO₂, H₂S Production Test (Harley and Prescott 2012, Brooks *et al.* 2017). The confirmed examination was performed via using API system (BioMerieux/France).

Antibiotic Sensitivity Test

To detect the *E. coli* sensitivity against Penicillin (10µ/disc), Tetracycline (30µ/disc), Amoxicillin (30µ/disc), Nalidixic acid (30µ/disc), Trimethoprim-Sulphamethoxazo (25µ/disc), Nitrofurantoin (300µ/disc), Gentamycin (10µ/disc), Nitrofurantoin and Chloramphenicol (30µ/disc). Antibiotic (Bioanalyse, Turkey) by using the disc diffusion method designated by Kirby (2011). 'The diameters of inhibition zone were measured and interpreted according to Clinical Laboratory Standard Institute (2011).

Standardization of Inoculum

The inocula were prepared from the stock cultures, which were maintained on nutrient agar slant at 4 °C and subcultured onto nutrient broth using a sterilized wire loop. The density of suspension inoculated onto the media for susceptibility test was determined by comparison with 0.5 McFarland standard of Barium sulphate solution (Cheesbrough *et al.*, 2015).

Preparation of Test Sample

In this study, concentrations of 400 mg/ml of the extracts were used to screen for the antimicrobial activity. This was done by using the modified method of NCCLS (2013). Here, 2.5 g of the extract was dissolved in each of the extracting solvents.

Antibacterial Assay

Medicinal plants using agar well diffusion method.

This was carried out by using agar well diffusion techniques. In this method, each of the labelled plates was uniformly inoculated with the organisms using pour plate techniques. A sterile cork-borer of 6mm diameter was used to make wells on the medium. 0.1ml of the various extract concentrations were dropped into each labelled well. After that, the plates were incubated anaerobically at 37 °C for 24 h. Antibacterial activity was

determined by measuring the diameter of zones of inhibition (mm) produced after 48 h of incubation. 0.05% Cephalosporin was used as control.

Determination of minimum inhibitory concentration (MIC)

Here, various concentrations of the extracts were obtained using double-fold serial dilution. Each dilution was assayed against the test bacterial using tube dilution techniques. One millilitre of test organism was added into each dilution incubated anaerobically at 37 °C for 24 h. The MIC was defined as the lowest concentration able to inhibit any visible bacterial growth. This was determined and recorded (Shahidi-Bunjar, 2014).

Acute Toxicity Studies

NRC guidelines were followed to carry out acute toxicity studies. All the animals of the group received single dose of hydroalcoholic extract (2000mg/kg p. o.) After administration of extract animals were observed continuously and individually for 30 minutes, 2 hours and 24 hours to detect changes in the autonomic or behavioural responses and also for tremors, convulsions and salivation.

Experimental Animals and Design

Twenty- five male albino rats of wistar strain weighing between 80-110g were obtained from the animal colony of Department of Biochemistry, University of Nigeria Nsukka, Enugu State, Nigeria. The animals were housed in a well-ventilated experimental animal house and were placed on pelletized commercial rat feed (Pfizer livestock Co. Ltd, Aba, Nigeria) and portable water *ad libitum*. They were left to acclimatize for five days. After acclimatization period, the rats were separated into five groups of five rats each. Their weights were equalised as nearly as possible. Aside the control groups, the remaining groups were given compounded feed and water for twenty-eight days.

Treatments for the rats were as follows;

to study the protective effect of hydroalcoholic extract of leaves of *Solanium aethiopicum* against *Escherichia coli*-induced pyelonesphritis, 48 male Wistar rats were randomly enrolled into 8 equal groups and treated as following: Group-I was kept normal by administering only vehicle. Group-II was kept as curative control and subjected to *Escherichia coli* administration at a

dose of 5mg/kg, b.w. intra-peritoneally (single dose). Group-III and Group-IV were administered orally *via* gavage with lower and higher doses of the aqueous extract *i.e.*, 200 and 400mg/kg, b.w. from day 5 to day 9. Group-V is the prophylactic control which administered with *Escherichia coli* on day 5. Group-VI and Group-VII were administered with lower and higher doses of the aqueous extract *i.e.*, 200 and 400mg/kg, b.w. from day 1 to day 5. In the curative regimen rats were initiated with intra-peritoneal injection of *Escherichia coli* at a dose of 5mg/kg,b.w. (single dose) on day 1 and in the prophylactic regimen *Escherichia coli* was administered on day 5 with one hour gap of oral administration of aqueous extract. Group-VIII served as standard which was administered with *Escherichia coli* on day 1 and Ciprofloxacin (5ml/kg, b.w.) from day 5 to day 9.

Blood Sample Collection

At the end of the treatment period, the rats were sacrificed by making incisions at their cervical regions with sterile blades after being put to sleep in a close container with help of chloroform. Their weights were also taken. Blood was collected by direct heart puncture with help of syringes into anticoagulant free tubes for haematology parameter studies.

Haematology Test

Blood percentage (Hb) and RBC levels were determined using Sahi's methods Alexander and Griffith [25] respectively. Westergreen's method was used for erythrocyte sedimentation rate (ESR), FACS methods were used for white blood cell total count (WBC Total) and differential counts respectively. Haematocrit method was used for packed cell volume (PCV) whereas, mean corpuscular volume (MCV) mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), were determined as described by Alexander and Griffith [26].

Result

The result of haematology analysis (Table 1) revealed that Hb (g/dl) levels increased from 12.23±0.17 to 15.07±0.20; PCV(%) ranged from 39.14 to 48.22±1.40;WBC(10⁹/L) ranged from 7.32±0.21 to 10.41±0.10; lymphocytes(%) ranged from 56.34±1.30 to 61.64±1.18; eosinophils(%) from 0.11±0.01 to 0.38±0.03; monocytes (%) ranged from 0.50±0.06 to 0.49±0.06; basophils (%) 0.30±0.08 to 0.35±0.03; MCH (pg) ranged from

3.52±0.10 to 4.10±0.39; MCHC (g/dl) ranged from 0.90±0.03 to 0.93±0.05 ;and ESR (mm/hr) ranged from 5.01±0.16 to 5.32±0.89.

The test organisms used for this study were isolated from urine sample of infected patients. Extract showed pronounced activities against the test organism. The ethanolic extract showed that as the concentrations of the extract increases, the antibacterial activity increases as indicated by increased in diameter zones of inhibition (Table 3). The leaf extracts inhibited *S. aureus* the most followed by *S. pneumoniae*, *H. influenzae* and

K. pneumoniae least. The inhibition produced by the leaf extracts differed significantly ($p \leq 0.05$) from that of the control antibiotics (Cephalosporin). The results of lung infection healing activity are showed in Table. The results showed that aqueous extracts of *Solanum aethiopicum* accelerate the progression of lung infection healing activity. As the concentrations of the extract increase, the rate of lung healing increase. Mice treated with the extracts showed considerable signs of diseases healing and significantly ($p \leq 0.05$ healed) earlier compare to control (water only).

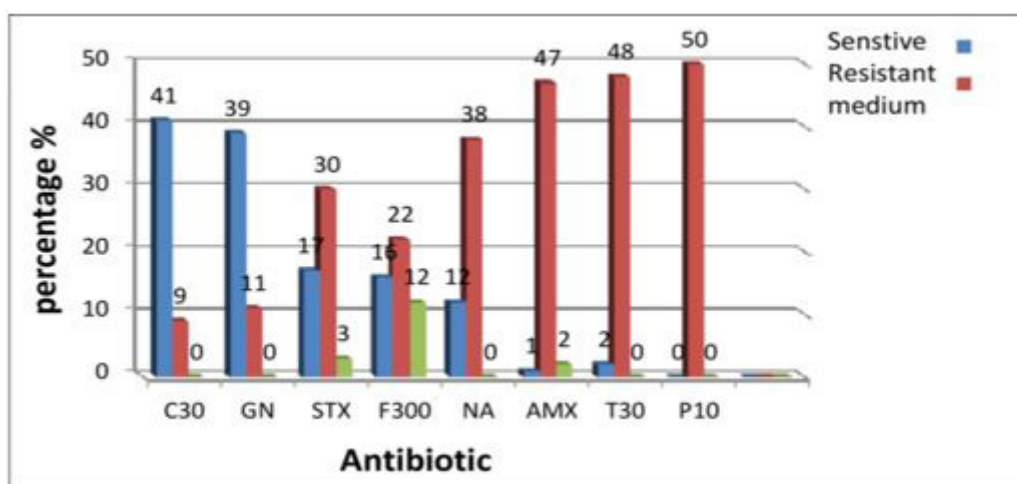


Figure 1: Antibiotic Sensitivity Test

Table 1: Acute toxicity studies of haematology of rats given *Solanum aethiopicum* leaf for 28 days

Groups					
Parameters	Control	I _a	I _b	I _c	I _d
Hb (g/dl)	12.23±0.17*	14.18±0.03	14.60±0.40*	15.01±0.93*	15.07±0.20*
PCV (%)	39.14± 1.10*	45.38± 1.05*	46.72± 1.00*	48.03±1.06*	48.22±1.40*
WBC (10 ⁹ /L)	7.32±0.21*	10.04±0.77*	10.20±0.60*	10.35±0.25*	10.41±0.10*
Lymphocytes(%)	56.34±1.30*	58.97±1.15*	60.04±1.29*	62.19±1.30*	61.64±1.18*
Eosinophils(%)	0.11±0.01	0.25±0.02	0.28±0.03	0.20±0.02	0.38±0.03
Monocytes (%)	0.50±0.06	0.41±0.01	0.43±0.09	0.44±0.08	0.49±0.06
Basophils (%)	0.30±0.08	0.31±0.01	0.33±0.01	0.35± 0.03	0.30±0.02
MCH (pg)	3.52±0.10	3.56±0.22	3.59±0.19	4.08±0.10	4.10±0.39
MCHC (g/dl)	0.90±0.03	0.92±0.01	0.91±0.02	0.93±0.01	0.93±0.05
ESR (mm/hr)	5.01±0.16	5.09±0.27	5.14±0.10	5.32±0.89	5.10±0.20

Results are mean and standard deviation of five determinations. Values asterisked are statistically significant against the control ($p < 0.05$).

Table 2: Mean diameter zones of inhibition of ethanol leaf extracts against the tested organisms (mm)

Concentration of extract (mg/ml)	<i>E.coli</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus sp</i>	<i>Klebsiella sp</i>
400	14.00	12.50	11.80	10.60
200	12.00	10.00	9.30	8.40
100	9.00	8.00	7.20	6.80
50	7.00	6.20	5.10	-
0.05%CEP	21.00	17.00	14.50	12.80

Table 3: Minimum inhibitory concentration (MIC) of the test extracts (mg/ml)

Extracts	<i>E. coli</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus sp</i>	<i>Klebsiella sp</i>
Ethanol	50	50	100	100
Aqueous	100	100	200	200

Table 4: The effect of *Solanum aethiopicum* leaf extract on *E.coli* in vivo

Treatments	T. WBC SX10 ³	NEX10 ³	Lyx10 ³	MOX10 ³	EOX10 ³
After zero time from infection A	4.60	3.05	1.17	0.37	0.00
B	4.30	2.79	1.07	0.34	0.00
AB	4.50	3.05	1.17	0.37	0.00
After 5 days from infection A	4.60	3.05	1.17	0.37	0.00
B	9.70	7.96	1.02	0.72	0.00
AB	11.30	10.05	1.18	0.10	0.00
After 8 days from infection A	4.60	3.05	1.17	0.37	0.00
B	14.40	7.13	0.61	0.69	0.00
AB	9.50	7.96	1.02	0.72	0.00
After 12 days from infection A	4.60	3.05	1.17	0.37	0.00
B	16.30	13.84	1.32	0.94	0.00
AB	7.30	6.31	0.09	0.58	0.00
After 16 days from infection A	4.60	3.05	1.17	0.37	0.00
B	13.60	11.85	1.63	0.21	0.00
AB	5.10	5.58	0.98	0.51	0.00

A = Control; B = Infected mice with *E.coli*; AB = Infected mice with *E.coli* and treated with plant extract; TWBCs = total white blood cells; Ne= Neutrophil; Ly = Lymphosite; Mo= Monosite Eo = Eosinophil

Discussion

There are many factors which are responsible for the kidney infections or damage such as hydrocarbons, chemical, drugs and microbes. The importance of haematology test in assessment of blood relating functions of substances that enter the body cannot be overstated [33-34]. Results of haematology as shown in Table 1, revealed that Hb levels increased apparently in test group 1a and became significant ($p < 0.05$) in test groups Ib,

Ic and Id when compared to the control. Increase in Hb level is normally followed by corresponding increase in PCV level. This could be the cause of the observed significant ($p < 0.05$) increase in PCV levels of test groups against the control in the present study. The increased Hb and PCV levels in this study could be that the studied fruit does not induce anaemia. The WBC and its differentials are known to protect the body against foreign body [35]. Their increase in the system is considered as defensive mechanism by the immune system [36]. Aside lymphocytes,

eosinophils, monocytes, and basophils were insignificantly ($p > 0.05$) affected in test groups when compared to the control. It could be that *Solanum aethiopicum* leaf induced the production of lymphocytes in test rats. MCH and MCHC are among the parameters used to determine the future of the body in terms of blood relating disease conditions. Both MCH and MCHC were insignificantly ($p > 0.05$) affected in test rats against those of the control. Their insignificant effect could be that consumption of *Solanum aethiopicum* leaf may not be linked with any future blood relating disease condition. The ESR is useful as a screening test for any acute or chronic infectious conditions with marked alteration in plasma protein concentration. Serial ESR can be used to monitor disease progression or treatment. Immunoglobulins are affected in raised ESR while increased plasma albumin slows the ESR [37]. The ESR levels in test groups were insignificantly affected ($p < 0.05$) when compared to the control group. It could be that the studied leaf did not affect the ESR of the test rats in the present.

The ethanolic leaf extract inhibited more than the aqueous leaf extract (Table 4). This could be due to the fact that ethanol is an organic and polar solvent and dissolve more of the active ingredients which are mainly organic in nature. The extracts inhibited *E. coli* most followed by *Staphylococcus aureus*, *Streptococcus sp* and *Klebsiella* was the least. This means that extracts could be easily used to manage enteric infections or any infection associated with *E. coli* or *S. aureus*.

The result revealed different symptoms of diseases in laboratory rats (*Mus musculus*) that were infected with 5×10^6 CFU/ML of *E. coli* isolate after 5 days of infection rats, such as swallowing, lung raised to out the body and they become weak and the total WBCs count raised from 4.5×10^3 cells to 11.3×10^3 cells/ μ l. The WBCs used as an immunological parameters to determine the case of infection (provan *et al.*, 2004), while normal range of total WBCs were 4.2×10^3 cells/ μ l (Hoffman *et al.*, 2000, Provan *et al.*, 2004), because the main type of phagocytic cells which is required to participate in the phagocytosis in the ingestion of foreign bodies (like bacterial cells) are neutrophil and macrophage (Kern, 2002 ; Henderson and Oyston, 2000; Ernst and Stendahl, 2006), so during infection with bacteria, the range of neutrophils increase comparing with control. While basophile and eosinophil are role model in immunity, eosinophil increasing in cancer and parasitic infections (Bain and flower, 1996). High level of basophils generally corresponds to an active allergic response (Wikipedia the free encyclopedia, 2010) [36-40].

After three days of administration, all the infected rats were examined to number of total WBCs and differential leukocyte count to check the effects of aqueous extracts at 400mg/ml, the total WBCs of infected mice were treated with sub- MIC only was 5.1×10^3 cells μ l and differential leukocyte count decreased when compared with control group.

At the end of the experiment, when the amounts were examined for TWBCs and other immunological parameters, the result indicated that for infected mice the total WBC raised to 16.3×10^3 , due to the effect of *Solanum aethiopicum* extract. During infection, all the infected mice and the control examined to total bacteria in the blood (table) represent count of viable bacteria presented in the blood.

After 5 days from infection, viable bacteria increased to 166 and 333 for infected with bacteria only and infected mice treated with plant extract respectively.

After 8 days from infection in the extract treated mice, bacterial number decreased considerably to 166, then to zero up to the end of experiment, while 5×10^2 and then increased to 1×10^3 cells ml, after 12 days, then decreased to 5×10^2 at the end of the experiment.

These findings have clearly demonstrated that the clearance of *E. coli* from the blood of infected mice by aqueous extract was zero, as compared with the infected untreated mice even after 16 days from infection the number was 5×10^2 cells μ l. Furthermore, it was more effective than other treatments.

The effect of *Solanum aethiopicum* extract may be due to that mkpuruofe is rich in tannin and other components and the antimicrobial activity of tannin is well documented (Chung *et al.*, 1998; Abu- Shanab *et al.*, 2005); Gulmez, *et al.*, 2006). The aqueous extract displayed broad spectrum of activity, i.e. G+ and G- bacteria were inhibited with *Solanum aethiopicum* extracts (Abu- Shanab *et al.*, 2005).

The results of MIC of the leaf extracts showed that the ethanolic and aqueous leaf extracts of *Solanum aethiopicum* possess antibacterial activity against *E. coli*, *S. aureus*, *Streptococcus sp* and *Klesiella sp*.

Conclusion

This study has shown that the phytochemicals and anti-bacterial constituents of *Solanum aethiopicum* can influence and restore cellular functions as well as structural integrity of the kidney. The results of the present study support the folkloric usage of the plant and suggests that medicinal plants may be a potential source of natural, safe and cheap in treating bacterial infection. The study revealed that the leaf extracts exhibited pronounced activities against the tested organisms.

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