NIPES

Journal of Science and Technology Research

Journal homepage: www.nipesjournals.org.ng

Exploring the Antibacterial and Haemo-Modulatory Effects of Aqueous *Hibiscus sabdariffa* Leaf Extract on *Escherichia coli*

Aziegbemhin, A. S.[1*] & Wemambu, E. C. [1]

[1]Department of Microbiology, Faculty of Life Sciences, University of Benin P.M.B1154 Benin City, Edo State Nigeria
*Email: abumhere.aziegbemhin@uniben.edu

Article Info

Keywords: *antibacterial, haemo-modulatory, hibiscus sabdariffa, leaf extract*

Received 5 March 2024
Revised 17 May 2024
Accepted 22 May 2024
Available online 30 June 2024

https://doi.org/10.5281/zenodo.12628737

Abstract

Hibiscus sabdariffa is commonly used for local beverages. This research investigated the in vivo antibacterial and immunomodulatory activities of aqueous Hibiscus sabdariffa leaf extract against Escherichia coli ATCC29214 in albino rats. Three groups of five rats each were used for this study. Groups B and C were orally given E. coli by gavage to induce gastrointestinal tract infection. Rats in Group C were treated with plant extract while those in Groups A were given commercial feed and clean water ad libitum. Stool samples obtained from the various groups were analyzed using standard microbiological protocols. Similarly, blood samples were also obtained from rats in the various groups for haematological analysis. The mean heterotrophic bacterial counts in the untreated group ranged from 64 x 10^6 cfu/g to 66 x 10^6 cfu/ml and 27 x 10^6 cfu/g to 6.0 x 10^6 cfu/g for treated rats respectively. Haematological parameters assayed varied between infected rats exposed to aqueous H. sabdariffa leaf extract and the unexposed group. Haemoglobin (g/dl) ranged from 14.7g/dl in the control through 13g/dl in the untreated and 15.3g/dl in the treated group. Procalcitonin levels in the untreated group was 577,000 (µl) 10^3 compared to 481,000 (µl) 10^3 and 471,000 (µl) 10^3 in the control and treated respectively. WBC count was 11,900 in the control group, 20,800 in infected rats and 10,300 in the treated group. The research shows that the aqueous H. sabdariffa leaf extract posses in vivo antibacterial activity and immunomodulatory actions that may be useful for combating gut infection.

1.0. Introduction

Escherichia coli is a Gram negative, bacilli in the Enterobacteriaceae family. There are hundreds of *E. coli* bacteria strains in the world. Most of them are harmless to humans, however some strains can cause infection in humans such as, bloody diarrhea, Urinary Tract Infection (UTI), hemolytic uremic syndrome and kidney failure. In some cases, these infections may lead to death. In recent decades, the increase of *E. coli* resistance to the β-lactams antibiotics such as penicillin, cephalosporin, monobactam and carbapenems has become more serious [1],[2]. Extended spectrum β-lactamase (ESBL) is the major reason for *E. coli* resistance to these antibiotic drugs [3].

Roselle (*Hibiscus sabdariffa* L) is an annual, erect, bushy, herbaceous sub-shrub that may grow to 8 ft (2.4m) tall, with smooth or nearly smooth, cylindrical, typically red stems. The leaves are alternate, 3 to 5 in (7.5 – 12.5 cm) long, green with reddish veins and long or short petioles. It is extensively cultured in tropical Africa, Asia, Australia and Central America [4].

Phytochemicals are bioactive compounds present in various plant components such as flowers, leaves, stems, roots, bark, and seeds. These non-nutritive substances play a crucial role in safeguarding human health by helping to prevent a wide range of degenerative diseases and pathological conditions [5]. In Nigeria, the hibiscus plant is commonly utilized in the creation of the well-loved Zobo drink. Research has demonstrated that this plant boasts a significant abundance of essential minerals and nutrients, including iron, copper, calcium, magnesium, and manganese. These components are crucial for promoting optimal human growth and overall health. [6].

Roselle is widely used for the treatment of diseases [7] used the aqueous methanolic extract of roselle to investigate its phytochemical constituents, antimicrobial activity and cytotoxicity, and reported that the extract contained cardiac glycosides, flavonoids, saponins and alkaloids. It exhibited antibacterial activities against *Staphylococcus aureus*, *Bacillus stearothermophilus*, *Micrococcus luteus*, *Serratia mascences*, *Clostridium sporogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus cereus* and *Pseudomonas fluorescence*.

Being a phyto-based therapy, it aligns with viable practices in the field of medicinal plants. Researchers may be able to rich harness the rich phytochemical assets of *H. sabdariffa*. They could also be able to investigate its potential as an effective antimicrobial and hemo-modulatory agent against *E. coli*. This approach not only aims to authenticate experience from folklore but also to add to the growth of newer treatment approaches combat microbial infection as well as mitigating antimicrobial resistance.

The usage of mathematical models as well as matching *in vitro* studies has can be attributed to low antibiotic levels [8] hence the need to undertake more *in vivo* experiments. The aim of this research was to assay for the antibacterial activity of *Hibiscus sabdariffa* against *Escherichia coli in vivo* as well as to determine its immunomodularoty properties using mouse model. The specific objective of the research work is to investigate the *in v*ivo antibacterial activity of extracts of *Hibiscus sabdariffa* using *Escherichia coli* in infected albino rats. Additionally, this research investigated some haematological indexes indicative of immunomodulation.

2.0. Materials and Methods

(a) **Animals**

Fifteen adult whistar rats weighing 120 to 135 grams were used for this study. The rats were purchased at the Department of Animal and Environmental Microbiology, University of Benin, Benin City, Nigeria.

(b)Test Bacteria and Confirmation

The test bacterial isolates used for this study was *Escherichia coli* ATCC29214 obtained from the Department of Microbiology, University of Benin, Benin City. Broth culture of bacterial isolate was prepared by using a sterile loop to obtain bacteria from a previously stored slants and inoculating same onto an already prepared sterile Eosine Methylene Blue Agar plate. After 24 hours of incubation at 37 ^{o} C colonies with metallic blue sheen characteristic of *E. coli* were seen on Agar plate. They were further confirmed by means of the morphological and biochemical characteristics [9].

(c) Plant materials and Extraction

Hibiscus sabdariffa leaves were purchased from New Benin Market, Benin City. The leaves were identified at the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. Plant materials collected was thoroughly washed under running tap water. The leaves were allowed to air dry at room temperature for fourteen (14) days. The dried leaves were grinded using electric blender into a powdery form. Fifty grammes (50g) of the pulverized leaves of *H. sabdariffa* was decocted with 250ml of water and left for 48 hours with occasional stirring. The mixture was then filtered and the filtrate evaporated to semi solid mass using a rotary evaporator [10].

(d) Preparation of Animals

Fifteen (15) female Wister mice used for this study were randomly assigned into three (3) groups, their age ranged between (6 – 7) weeks with weight ranged between (100g – 120g). These were maintained in the animal house of Microbiology Department, University of Benin, Benin City. They were put in standard cages with food and water and at optimum conditions of temperature, light and ventilation and were acclimatized for 2 weeks before infection and administration of the extracts commenced. The cages were cleaned every morning and disinfected at three days interval.

(e) ***In vivo*** **Assay using Wister rats**

Wister rats were randomly divided into the following group; Control (CON), *Escherichia coli* infected (EI) and *Escherichia coli* infected + *Hibiscus sabdariffa* (EIHS). Rats in this group were not induced with *Escherichia coli* and were fed with normal rat chow and had free access to water throughout the period of the experiment. They were used to monitor successful induction of diarrhea (CON). Rats in EI group were fed with normal rat chow and orally given a single infectious dose (2×10^8 cfu/ml) of *Escherichia coli* but were not treated with the extract. In the EIHS group, rats were fed with normal rat chow and orally given a single infectious dose of *Escherichia coli*. After infection, the animals were orally given 750mg/kg aqueous extract of *Hibiscus sabdariffa* extract daily for five (5) days.

2.1. Collection and Preparation of Blood Samples for Analysis

Twenty-four (24) hours after the last treatment was given, all the rats were weighed and sacrificed under chloroform vapour anesthesia. Five (5) ml of blood were collected from each animal by cardiac puncture into EDTA treated screw-cap sample bottles. Samples were used for hematological analysis.

2.2. Haematological Analysis

Full Blood Counts such as Packed Cell Volume (PCV), Red Blood Cells (RBC), Haemoglobin (Hb) Total White Blood Cell (TWBC), Platelet count and differential White Blood Cell (lymphocytes, monocytes, neutrophils, eosinophils) were estimated using the Automated Haematological Analyzer KX-ZIN (Sysmex, Japan). Mean Cell Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC) and Mean Corpuscular Hemoglobin (MCH) were calculated.

2.3 Statistical Analysis

All data were analyzed using Statistical Package for Social Sciences (SPSS) version 4.3.1.Mean and Standard deviation for all bacterial counts were calculated.

3.0. Results

Table 1 shows the mean *E. coli* counts of faecal matter of the control and untreated and treated rats. In the control group, bacterial counts ranged from $17 \pm 2.83 \times 10^6$ cfu/ml at day 1 to $28 \pm 5.66 \times 10^6$ cfu/ml

at day 3. Bacterial counts in the untreated rats ranged from 64±9.19 x 10^6 cfu/ml to 66±9.98 x 10^6 cfu/ml from day one to day three.

Results of the haematological parameters were presented in Table 2. Haemoglobin level was observed to increase from 13g/dl in untreated rats to 15.3g/dl in treated rats. The RBC decreased from 7.1 x 10^{12}/L in control rats to 6.1 x 10^{12}/L in untreated rats and then increased to 7.3 x 10^{12}/L in treated rats. PCV values decreased from 45% in the control to 42% in the untreated rats and increased to 56% in treated rats.

The White Blood Cells profiles of aqueous *H. sabdariffa* leaf extract was shown in Table 3. The neutrophils increased from 9 in the control to 12 in the untreated rats and 19 in the treated rats. The White Blood Cells reduced from 11,900 to 20,800 in the control and untreated rats respectively and then increased to 10,300 in the treated rats. The lymphocytes increased from 80 in the control rats to 83 in the untreated rats and reduced to 80 in treated rats. Eosinophil increased from 1 in the untreated rats to 2 in the treated rats. It remained at 1 in both control and the untreated rats. There was a reduction in the monocytes from 10 in the control rats to 3 in the untreated rats and then increased to 180 in the treated rats.

Table 4 depicts the results of the erythrocyte indices (MCV, MCH and MCHC). The MCV increased from 83fL in the control to 85fL in the untreated and then decreased to 71fL in the treated rats. MCHC remained at 21 x 10^{-12} in the control and untreated rats but decreased from 21 x 10^{-12} to 19 x 10^{-12} in untreated and treated respectively.

3.1. Discussion

The antibacterial properties of plants in general have been attributed to the presence of phytochemicals such as flavonoids, alkaloids, tannins, saponins and terpenes in plants. Flavonoids are known to be synthesized by plants in response to microbial attack. Their activity is due to their ability to react with extracellular and soluble proteins and to complex with bacterial cell walls leading to the death of the bacteria [11],[12]. Tannins are also reported to have various physiological effects like anti-irritant, antisecretolytic, antiphlogistic, antimicrobial and antiparasitic effects. Phytotherapeutically, tannin containing plants are used to treat nonspecific diarrhea, inflammations of mouth and throat and slightly injured skin [13].

E. coli counts of faecal matter of albino rats infected was observed to be higher when the treated rats with *H. sabdariffa*. *E. coli* counts of faecal matter in the untreated rats ranged from 64 x 10^6 cfu/ml to 66 x 10^6 cfu/ml from day one to day three, while it ranged from 27 x 10^6 cfu/ml to 60 x 10^6 cfu/ml. Counts were observed to be increasing from day one to day three in the untreated rats. However, it was reduced in rats treated with the plant. The higher count observed on day 1 was due to low concentration of the plant extract in the rats, but during prolonged period, the plant extract circulated in the rats and drastically reduced *E. coli* counts of faecal matter of the rats. Reduction in *E. coli* counts in rats treated with *H. sabdariffa* shows potential in vivo antibacterial activities of the plant. This agrees with the study of [14] who also found that *E. coli* counts reduced after several days of administration. While ours went from 64±9.19 to 63±8.48 in day 1. To the best of our knowledge, there seem to be no discordance with our findings.

Table 1: Mean *E. coli* counts in feacal matter of infected rats treated with extract of *H. sabdariffa*.

	Control	Untreated	Treated
Days	**Mean**	**Mean**	**Mean**
1	17±2.83	64±9.19	63±8.48
2	25±5.00	65±9.19	36±7.07
3	28±5.66	66±9.89	27±4.24

Table 2: Hematological profiles of aqueous *H. sabdariffa* leaf extract on albino rats

	Control	**Untreated**	**Treated**
Hb (g/dl)	14.7	13	153
RBC (10^{12}/L)	7.1	6.1	7.3
Procalcitonin (µl) 10^3	481,000	577,000	471,000
PCV (%)	45	42	56

Key:
Hb = Haemoglobin
PCV = Packed Cell Volume RBC = Red Blood Cells

Table 3: White Blood Cells profile of aqueous *H. sabdariffa* leaf extract on albino rats

	Control	**Untreated**	**Treated**
Neutrophils	9	12	19
WBC	11,900	20,800	10,300
Lymphocytes	80	83	80
Eosinophils	1	1	2
Monocytes	10	3	180

Key:
WBC = White Blood Cells

Tab**le 4:** Erythrocytes indices of aqueous *h. sabdariffa* leaf extract on albino rats

	Control	**Untreated**	**Treated**
MCV (fL)101^5	83	85	71
MCHC (g/L)	250,000	250,000	273,000
MCH (10^{-12})	21	21	19

Key:
MCV = Mean Corpuscular Volume
MCHC = Mean Corpuscular Haemoglobin Concentration MCH = Mean Corpuscular Haemoglobin

Haematological parameters including white blood cell count, haemoglobin, packed cell volume, neutrophil, monocyte, eosinophil, procalcitonin, red blood cell and mean corpuscular volume, were observed to vary in *Escherichia coli* infected albino rats exposed to *H. sabdariffa* leaf extract as compared to the control group. Increase in haemoglobin enhanced the oxygen transporting properties of the red blood cells and is because of increased number of red cells, polycythemia which is usually caused by dehydration produced by *E. coli* infection. A low PCV of 42% initiated by *E. coli* infection was drastically increased to 56% when infected rats were given plant extract. The observed increase in the PCV recorded in this study on the administration of extract of *H. sabdariffa* may have been due to effect on bone marrow stem cell by improving its proliferative activity. Our work is in agreement with that of [15] who reported a similar finding.

The rise in white blood shows that *H. sabdariffa* has potential immunomodulatory properties, being able to engineer the immune system to fight against microbial infection. From this study, it shows that *E. coli* infection resulted to leucopenia in infected rat. This agrees with earlier work by [16] who reported the immunomodulatory activity of *H. sabdariffa* extract on splenic and thymic cells using mouse model. [15] also reported an immunomodulatory activity of aqueous and alcoholic extract of *H. sabdariffa*.

The number of neutrophil was observed to increase when infected rats were treated with the leaves extract as compared to untreated rats. Infection with *E. coli* led to an increase in neutrophil counts. On exposure to plant extract, there was hypermodulation of neutrophil, thus helping to reduce microbial load by active phagocytosis [17]. Procalcitonin level usually increases as a result of pro-inflammatory response due to bacteria origin. *E. coli* infection in rats increased PCV level from 481,000 to 577,000. However, after treatment with *H. sabdariffa* leaves, the value was reduced to 471,000. It is clear from this experiment that *H. sabdariffa* does play an immune regulatory function *in vivo*, helping the host animal boost its immune efficacy to fight against infection [5].

The reduction in the mean cell volume signifies that the size of the RBC was reduced, indicating microcytic anemia due to either iron deficiency anemia and/ anemia of chronic disease [18]. Reduction of the mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) supported the possibility of the extract predisposing the consumer to iron deficiency anemia and/or microcytic hypochromic anemia [19].

[20] had a contrary report. Their study however indicates that extract of *H. sabdariffa* could have erythrocytes building capacity due to high level of iron.

4.0. Conclusion

The research conducted demonstrates that the extract of *H. sabdariffa* exhibits promising in vivo antibacterial effects against *Escherichia coli* ATCC29214. Additionally, it indicates that *H. sabdariffa* possesses potent immunomodulatory properties which enhance the immunity of afflicted host organisms, empowering them to combat infections effectively. The extract of *H. sabdariffa* presents itself as a potential substitute for traditional antibiotics in the management of *E. coli* infections. Despite these promising findings, further studies are imperative to determine its toxicity profile and identify the specific phytochemical constituents responsible for its antibacterial efficacy as well as it mechanism of action.

References

[1] Mączyńska, B., Frej-Mądrzak, M., Sarowska, J., Woronowicz, K., Choroszy-Król, I. and Jama- Kmiecik, A. (2023). Evolution of Antibiotic Resistance in *Escherichia coli* and *Klebsiella pneumoniae* Clinical Isolates in a Multi-Profile Hospital over 5 Years (2017-2021). *Journal of Clinical Medicine* 12, 2414. https://doi.org/10.3390/jcm12062414

[2] Abhijit, A., Sunita, N. and Maria K. S. (2013). Study of urinary isolates with reference to extended spectrum beta lactamases detection and antibiogram. *Journal of Evolution of Medical and Dental Sciences* **2** (9):1049–1055.

[3] Dubreuil, J. D. (2014). The Whole Shebang: The Gastrointestinal Tract, Eschericia coli Enterotoxins and Secretion. *Current Issues in Molecular Biology* **14**:71-82.

[4] Afari-Sefa, V., Rajendran, S., Kessy, R. F., Karanja, D. K., Musebe R., Samali, S. and Makaranga, M. (2016). Impact of Nutritional Perceptions of Traditional African Vegetables on Farm Household Production Decisions: A Case Study of Smallholders in Tanzania. _Experimental Agriculture_ *52* (2): 300-313.

[5] Kumar, M., Garg, R. and Garg, R. (2023). Phytochemical Properties and Antioxidant Activity of Hibiscus sabdariffa Linn. *International Journal of Pharmaceutical and Chemical Sciences* **1**(3):1236-1241.

[6] Okereke, C. N., Iroka, F. C. and Chukwuma, M. O. (2015). Phytochemical analysis and medicinal uses of Hibiscus sabdariffa. *International Journal of Herbal Medicine* **2**(6):16-19.

[7] Sehim, A.E.; Amin, B.H.; Yosri, M., Salama, H. M., Alkhalifah, D. H., Alwaili, M. A. and Elghaffar, R. Y. (2023). GC-MS Analysis, Antibacterial, and Anticancer Activities of Hibiscus sabdariffa L. Methanolic Extract: In Vitro and In Silico Studies. _Microorganisms_ *11* :1601. https://doi.org/10.3390/microorganisms11061601

[8] Neil, K., Allard, N. and Grenier, F.(2020). Highly efficient gene transfer in the mouse gut microbiota is enabled by the Incl2 conjugative plasmid TP114. *Commun Biol* **3**:523. https://doi.org/10.1038/s42003-020-01253-0

[9] Lupindu, A. M. (2017). Isolation and Characterization of *Escherichia coli* from Animals, Humans, and Environment. *InTech* doi: **10**:5772/67390

[10] Adamu, H. and Ngwu, R. O. (2015). Phytochemical Screening And Antibacterial Activities Of Hibiscus Sabdariffa L. Leaf Extracts. *Nigerian Journal of Chemical Research* **20**:46-52.

[11] Alemu, M., Lulekal, E., Asfaw, Z., Warkineh, B., Debella, A. and Abebe, A. (2024). Antibacterial activity and phytochemical screening of traditional medicinal plants most preferred for treating infectious diseases in Habru District, North Wollo Zone, Amhara Region, Ethiopia. *PLoS ONE* **19**(3):030-060. https://doi.org/10.1371/journal. pone.0300060

[12] Othman, L., Sleiman, A. and Abdel-Massih, R. M. (2019). Antimicrobial Activity of Polyphenols and Alkaloids in Middle Eastern Plants. *Frontiers in microbiology* **10**:9-11. https://doi.org/10.3389/fmicb.2019.00911

[13] Mohammed, M. Y., Idris, I., Gandu, M. and Tanko, A. A. (2019). Phytochemical and Antimicrobial Study on the Leaf Extract of Tapinanthus dodoneifolius (Van Teigh) Loranthaceae. *Journal of Advances in Medicine and Medical Research* **29**:1-9.

[14] Portillo-Torres, A. L., Aurea, B. N., José, M. M., Carlos, A. G., Leopoldo, G. C., Esmeralda, R. V. and Javier, C. R. (2022). Antimicrobial Effects of Aqueous Extract from Calyces of Hibiscus sabdariffa in CD-1 Mice Infected with Multidrug-Resistant Enterohemorrhagic Escherichia coli and Salmonella Typhimurium. *Journal of Medicinal food* **25**(9):29-63. https://orcid.org/0000-0001-2911-7863

[15] Hussein, M.E., El-Senousy, A. S., El-Askary, H. I., Mouneir, S. M. and El-Fishawy, A. M. (2019). Immunomodulatory and anti-inflammatory activities of the defatted alcoholic extract and mucilage of Hibiscus sabdariffa L. leaves and their chemical characterization. *Journal of Phamacognosy & Phytochemistry* **8**(4):982-990.

[16] Umeoguaju, F. U., Ephraim-Emmanuel, B. C., Uba, J. O., Bekibele, G. E., Chigozie, N., Orisakwe, O.E. (2021). Immunomodulatory and Mechanistic Considerations of Hibiscus sabdariffa (HS) in Dysfunctional Immune Responses: A Systematic Review. *Front Immunol* **12**:550-670. doi: 10.3389/fimmu.2021.550670. eCollection 2021.PMID: 34040600

[17] Bokaeian, M., Sheikh, M., Shahi, Z. and Saeidi, S. (2014). Antimicrobial activity of Hibiscus sabdariffa extract against himan pathogen. *International journal of Advanced Biological and Biomedical Research* **2**(2):433-439.

[18] Sachdev, V., Rosing, D. R. and Thein, S. L. (2021). Cardiovascular complications of sickle cell disease. *Trends Cardiovasc Med* **31**(3):187-193.

[19] Karen, J. T. (2012). Review of Laboratory and Diagnostic Tests. *Clinical Skills for Pharmacists* **3**:86-122.

[20] Oyewo, B. and Akanji, M. I. (2019). In vitro and in vivo Evaluation if the Antioxidant Properties of Aqueous Extract of Andrographispaniculata Leaves. *Researcher* **2**(11):42-51.

[21] Kumar, A. P, N., Kumar, M., Jose, A., Tomer, V., Oz, E., Proestos, C., Zeng, M., Elobeid, T., K, S., and Oz, F. (2023). Major Phytochemicals: Recent Advances in Health Benefits and Extraction Method. *Molecules* **28**(2):808-887. https://doi.org/10.3390/molecules28020887

[22] Salem, M. Z. M., Perez, J. O. and Salem, A. Z. M. (2014). Studies on biological activities and phytochemicals composition of Hibiscus species – A review. *Life Science Journal* **11**(5):1- 8.