International Journal of Scientific Research in Multidisciplinary Studies
Vol.7, Issue.8, pp.43-48, August (2021)

Research Paper
E-ISSN: 2454-9312
P-ISSN: 2454-6143

# Antibacterial In-Vitro Evaluation of Phenotypically Screened Solasodine from *Solanum nigrum Linn.* Against Enterohemorrhagic *Escherichia coli* (0157:H7)

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**Available online at: www.isroset.org**

Received: 14/Aug/2020, Accepted: 20/Aug/2020, Online: 31/Aug/2021

***Abstract***—Many strains of *Escherichia coli (E.coli)* are not detrimental yet some can cause serious illness to humans. One virulent strain of this bacterium is 0157:H7 otherwise called as the Enterohemorrhagic *E.coli*. Since *E.coli* strains cannot be treated with the available synthetic antibacterial medicines due to its recorded increasing resistance, the quest for using alternative medicines has flooded the literature to treat *E.coli*-related infections. This study aimed to evaluate the antibacterial efficacy of phenotypically screened solasodine from *Solanum nigrum Linn*. through in vitro assay against *E.coli* 0157:H7. Disc diffusion method was employed for the antibacterial testing of isolated 97% ± 0.18% concentration of solasodine (experimental) in comparison to the 100% commercialized solasodine (positive control), and disc treated with 80% dried methanol (negative control). Results revealed statistical no difference ($p$>0.05) in the measure of zone of inhibitions between the positive control and experimental set-up across trials. The result is further supported by the consistency of data across trials ($p$<0.05), and no appearance of zone of inhibition in the negative control set-up. The in vitro testified for the antibacterial effectiveness of solasodine isolated from *Solanum nigrum Linn.* extract as an alternative organic source for combating infections caused by *E.coli* 0157:H7. Nevertheless, further analysis of *Solanum nigrum Linn*. extract has to be studied to determine its effect in other tissues through in vivo assay.

***Keywords***—Alternative medicine, Antibacterial testing, Escherichia coli, Phenotypic Screening, Phytopharmacology, Solasodine

## I. INTRODUCTION

*Escherichia coli (E.coli*) has hundreds of documented strains living in the gut of both animals and humans. Each strain has a specific virulence characteristic that is often plasmid-mediated [1]. Nevertheless, most of its documented strains are harmless. Enterohemorrhagic *E.coli* (0157:H7) is a virulent strain of *E.coli* capable of producing Shiga toxin that can severely damage the intestinal linings of humans resulting to bloody diarrhea with severe abdominal pain [2]. Its transmission is caused by eating undercooked beef while some accounts relate its outbreak on drinking contaminated water, and contact to animals [3]. In fact, reference [4] cited the 1993 outbreak of *E. coli* 0157:H7 in a large multistate case where it was associated to undercooked beef patty sold by a fast-food restaurant. Though *E.coli* 0157:H7 was first discovered in 1982, its first known outbreak occurred in Michigan, USA when cases of bloody diarrhea were reported [5]. Since then, *E.coli* 0157:H7 has been considered as an important foodborne and waterborne pathogen.

The startling thing about *E.coli*-related infection is how little medical professionals know about treating it [6]. Basically, rest and taking in plenty of fluids were suggested to prevent dehydration and fatigue. Synthetic antibiotics are not administered to treat infections caused by *E.coli* because their resistance is steadily increasing since its first reported case thus, imposing threat to humans [7]. Antibiotic abuse was seen to be the root cause of antibiotic-resistance of many bacteria pathogens inducing the race for developing and discovering other sources of alternative antibiotics to treat bacterial infections [8]. The pool of literature has documented studies on the effectiveness of organic antibacterial phytochemical sources *in vitro* against *E.coli*. One report on the successful use of alternative antibacterial agent was cited by reference [9] who noted that extracts of certain fruits and onion bulb can inhibit *E.coli* growth. Though some of those published papers have not specified the *E.coli* strain used, the study of reference [10] proved that the isolated solasodine was effective against *E.coli* (unspecified strain) and other wound-related bacteria. The study testified for the credence of using solasodine as an antibacterial agent.

## II. RELATED WORKS

According to reference [11], solasodine is considered a poisonous chemical that can be derived from tomatoes and potatoes but can be commercially processed for developing

 

steroids and contraceptive pills. Because of its steroidal property, the chemical seems to contain an immunosuppressive characteristic based from restrained number of lymphocytes in an *in vivo* study using mouse [12]. More importantly, the use of solasodine showed potential damaging effects in combating proliferative-stage of colorectal cancer cells in an *in vitro* study [13]. Reference [14] attributed the antibacterial effectiveness of solasodine against *Staphylococcus aureus* to its ability of destroying DNAs. Reports have also shown that solasodine has effects in the reproductive system following the *in vivo* experiments. Antispermatogenic property was observed in male dogs where the chemical solasodine reduced the levels of epididymides [15]. The chemical also shows a promising impact in lowering high bold pressure due to its antiantherosclerosis properties [16]. There is also a documented study that the chemical is capable of antifungal ability as used against *Candida albicans* [17]. There are also studies showing its efficacy as an antiviral agent against herpes [18]. Reference [19] summed up the conclusive effects of solasodine as anticancer, cardiotonic, antispermatogenic, antiandrogenic, immunomodulatory, antifungal, antipyretic, diuretic, and other significant effects especially in the central nervous system.

Recent reports have shown that solasodine is primarily extracted from solanum plant varieties [20][21]. Various methods of phenotypic screening were documented in measuring and validating the isolated solasodine from the plant extract. Reference [22] developed a HPLC-DAD method for the estimation of solasodine from the extract of a solanum species. Application of concomitant extraction process and hydrolysis was also conducted to extract the pure solasodine compound from *Solanum khasianum* for the development of steroids [23]. On the other hand, an environment friendly procedure of solasodine extraction was also conducted from dried plant material of *Solanum laciniatum* Ait. [24].

These reviews of works indicated the potentiality of isolating the pure solasodine through phenotypic screening, and its efficacy as an antimicrobial agent.

In this study, the use of solasodine isolated from the pure extract of *Solanum nigrum Linn.* was tested against the *E.coli* 0517:H7 *in vitro*. Specifically, this study aims to determine the (a) measure of zone of inhibitions of phenotypically screened solasodine against cultured *E.coli* 157:H7, and (b) determine the significance of difference in the measure of inhibitory zones between the isolated solasodine vis-à-vis the commercialized solasodine.

## III. METHODOLOGY

The aim of the study is to test the antibacterial efficacy of the isolated solasodine from *S. nigrum Linn*. through disc diffusion method. The successive narrations were the materials and procedures for the phenotypic screening and antibacterial testing of phytochemical solasodine.

**Materials**

Isolation of the solasodine content used *Solanun nigum Linn*. collected from *Cagraray*, Bacacay, Albay, Philippines. The plant material was identified and verified authentic species by SciTech Innovations, Department of Biology. The chemical consumables used for the isolation of solasodine were 2% aqueous oxalic acid, 60% NaOH, 0.5 M HCl, and 80% ACS grade methanol. Laboratory materials include magnetic stirrer apparatus, whatman no.1 filter paper, centrifuge, bunsen burner for heating, medicine dropper for transferring minute liquid volumes, digital thermometer for temperature monitoring, beakers for boiling, test tubes and test tube racks for containing, stirring rods for mixing, tripods, analytical balance for weighing materials, and graduated cylinder for measuring liquid volumes.

For the experimentation proper, materials include the phenotypically screened solasodine from *S. nigrum Linn.*, a commercialized 100% solasodine supplied by M.Rivera Clinical Analytics, 6.3 x $10^5$ CFU/ml inoculum size of *E.coli* 0157:H7 strain from SGS Microbial Testing for Life Sciences, Leeming-Notman agar as culture medium, petri dishes with discs for diffusion test, 80% ACS grade methanol to dissolve the powdered solasodine, test tubes and test tube racks for containing the mixtures, laboratory incubator for regulating temperature of culture, and caliper for measuring the inhibitory zones (mm).

Common laboratory autoclave sterilization of glasswares for 30 minutes at 15 psi in 12 ˚C was conducted before the experimentation to assure the reliability of the results by terminating extraneous variances like microbial contamination.

**Phenotypic Screening of Pure Solasodine from *Solanum nigrum Linn*. Extract**

The method for Isolating the solasodine from *S. nigrum Linn*. was adapted and modified from reference [25]. This was done by adding 50 ml of 2% aqueous oxalic acid to every 1 gram of dried and powdered leaves of *S. nigrum Linn.* The mixture was gently shaken for 30 minutes in a magnetic stirrer apparatus. Whatman no.1 filter paper was used to vacuum filter the suspensions where 25 ml of the clear extract filtrate was collected. The filtrate was gently heated at 75˚C and added with 1ml of 60% NaOH. The mixture was allowed to settle overnight at room temperature.

The mixture was centrifuged at 20˚C for 10 minutes at 3000rpm. Supernatants were removed by decantation. The suspended pellets were collected and dissolved in 5ml of 0.5 M HCl. The pellet mixture was hydrolyzed and refluxed at 100˚C for 90 minutes. After which, 1ml of 60% NaOH was added to the mixture. It was heated again to 100˚C for 10 minutes for the formation of solasodine insoluble. Eighty percent of ACS grade methanol was added to the compound to allow crystallization by drying at room temperature. The method for isolation of solasodine from *S. nigrum Linn*. was conducted in 3 trials. An average of 8.87 grams of dried green-white powder was

 

obtained from 50 grams of dried and powdered leaves. The isolated samples from all trials were sent to Sci&Tech Innovations for chemical analysis of the solasodine content. Result showed 97% ± 0.18% of the isolated sample was solasodine material. Table 1 was the result of chemical analysis.

Table 1. Chemical analysis report of solasodine content from 3 samples produced in trials.

| Samples from 3 Trials | Content (%) | sd |
|---|---|---|
| Sample 1 | 96.88 | |
| Sample 2 | 97.22 | 0.18 |
| Sample 3 | 96.94 | |
| Mean | 97.01 | |

Figure 1 below is the molecular structure of chemical solasodine

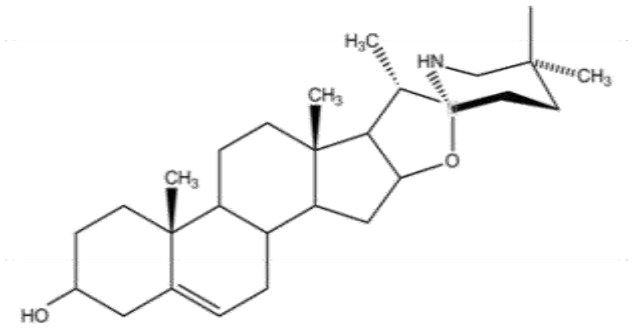


Figure 1. Molecular Structure of Solasodine [26].

**Antibacterial Screening of Solasodine Through Disc Diffusion Method**

The evaluation of antibacterial efficacy of isolated solasodine was through in vitro test using disc diffusion method into two set-ups; (1) experimental and (2) controlled. Preparation of the experimental set-up includes soaking of disc in the dissolved 4 grams of powdered solasodine by 4 ml of 80% ACS grade methanol. The disc was allowed to dry at room temperature until the alcohol has completely evaporated. The controlled set-up was prepared by soaking the disc in 4 drops of 100% commercialized solasodine supplied by M.Rivera Clinical Analytics (positive), while another controlled set-up soaked another disc into 4ml of 80% ACS grade methanol (negative). Likewise, the discs were also allowed to completely dry at room temperature. Sterilized Leeming-Notman agar in petri dishes was used as culture-medium for both set-ups. An inoculum size of 6.3 x $10^5$ CFU/ml of *E.coli* 0157:H7 strain from SGS Microbial Testing for Life Sciences was swabbed in the set-ups. The treated discs (experimental, controlled-positive, controlled-negative) were positioned at the center of the culture medium in the petri dish. Incubation period took 5 days for 37 ˚C until inhibitory zones became visible. Caliper was used to measure the zone of inhibition using millimeter (mm) as unit. The method was repeated for 3 consecutive trials to determine the consistency of results.

**Data Analysis of Results**

Descriptive and inferential statistics were used in the study. Specifically, mean, Analysis of Variance (ANOVA), and post-hoc analysis through *Bonferroni* correction were utilized.

Mean was employed to compute for the average measure of the zone of inhibition in 3 trials. To determine the significance of the difference across trials and set-ups, ANOVA was executed to treat the multiple sets of data using the measures of inhibitory zones (for set-ups), and consistency of results (for trials). This was supported by *Bonferroni* correction to further dig down on the data set that caused significant difference in ANOVA testing. Since 3 data sets comprise both the set-ups (experimental, positive control, negative contro), and trials (3 trials), 3 comparative events were expected in the *Bonferroni* test. Therefore, an alpha value of 0.016 was used in the post-hoc analysis.

## IV. RESULTS AND DISCUSSION

The diffusion method took 15 days to finish the 3 trials. Following the established research goals, the following narrations were the significant findings of the study.

**Measure of the Inhibitory Zones of Set-ups**

The data revealed vivid inhibitory zone in the isolation of solasodine from *S. nigrum Linn.* across the trials (8.17mm ± 0.29mm) (disc diameter subtracted). This result indicated that the phenotypic screening of solasodine derived from *S. nigrum Linn*. was found effective against *E.coli* 0157: H7. Table 2 shows the tabulated result on the measure of inhibitory zones after the periods of incubation.

Table 2. Measure of inhibitory zones between experimental and control set-ups across trials.

| | Measure of the diameter of zone of inhibition (mm) | | | Mean |
|---|---|---|---|---|
| | Trial 1 | Trial 2 | Trial 3 | |
| Experimental | 8.0mm | 8.5mm | 8.0mm | 8.17mm |
| Positive control | 9.0mm | 8.0mm | 8.5mm | 8.50mm |
| Negative control | 0.0mm | 0.0mm | 0.0mm | 0.0mm |

Although the data showed that positive control set-up showed the maximum antibacterial efficacy (8.5mm ± 0.50mm), the mean difference of 0.33 is statistically not significant ($p$>0.05) to the experimental group suggesting similarity in the results. This reveals that the use of *S. nigrum Linn.* extract, at desired quantities, can be used as an effective organic substitute to the commercialized 100% solasodine. Nevertheless, the minute discrepancy in the cast of mean supports the result of the previously conducted chemical analysis at the level of purity of the

 

isolated solasodine equivalent to 97% ± 0.18%. This further suggests that a minute marginal error in the result of experimental set-up had decreased the zone of inhibition due to isolated impure solasodine. It is assumed that the zone of inhibition in the experimental set-up is larger than the actual measure shown in the testing, which could have lesser mean difference than that of positive control set-up. Nevertheless, the mean difference of experimental and positive set-up showed no statistical difference. The antibacterial efficacy of the solasodine was deemed lethal for damaging the DNA of *E.coli* as supported by the reviewed literature. To visualize the result of in vitro testing, Figure 2 is presented.

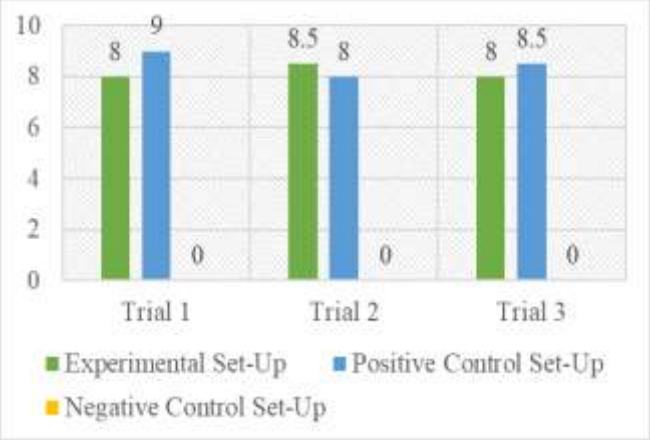


Figure 2. The measure of inhibitory zones for every set-up across trials.

The same result of antibacterial efficacy of solasodine against *E.coli* was documented in the body of literature implying its effectiveness against *E. coli*, and other bacteria. Though variation in the quantitative results occur, the discrepancy was associated to the diverse methodologies, techniques, and solasodine concentration employed by the researchers in their set-ups.

The in vitro testing result was further supported by the negative control set-up exhibiting no zone of inhibition across trials. This suggested that the inhibitory zones in experimental and positive control set-ups were caused by the solasodine without any extraneous variances that may alter the result of the study (e.q. the methanol remains in the disc as tested by the negative control). The in vitro testing of the phenotypically screened solasodine proved testimony to its effectiveness against the lethal *E.coli* 0157: H7 strain bacteria. Furthermore, antibacterial screening of the isolated solasodine against *E.coli* provided favorable result for treating infections caused by the bacteria. Thorough analysis needs to be conducted considering the isolated solasodine has other antibacterial phytochemical agents that might have possibly contributed in the result of inhibitory zones. This was assumed due to 3% phytochemical constituents of the isolated material (97% ± 0.18% only of solasodine content based from chemical analysis) which might be composed of other strong phytochemicals showing antibacterial efficacy. Nonetheless, proper screening of the solasodine content of *S. nigrum Linn*. can possibly lead to the development of innovative pharmaceutical drugs.

This study recommends to test other methods on isolating the pure solasodine to verify the result of the present study.

**Comparative Analysis of Results Across Set-ups and Trials**

Analysis of Variance (ANOVA) was conducted to determine the significance of difference among set-ups and trials.

Statistical test comparing data results among set-ups showed statistical difference in the measure of zone of inhibitions ($F(2,6) = 625.75$, $p<0.05$). This statistical result suggests that the measures of inhibition in all set-ups do vary, with varying levels of effectiveness against *E.coli* 0157:H7. Moreover, this result could also imply that at least 1 data set has caused the significant difference in the result of ANOVA testing. Therefore, a follow-up statistical measure was performed through a post-hoc analysis via *Bonferroni* correction to validate the assumptions of ANOVA test result. Bonferroni correction ($p=0.016$) showed that negative control set-up lied significantly different from the cohort of data ($p<0.016$). Meanwhile, the post-hoc analysis showed no statistical difference ($p>0.016$) between experimental and positive control set-ups' measures of zone of inhibition. The post-hoc analysis indicated that the isolated 97% ± 0.18% concentration of solasodine from *S. nigrum Linn.* is as effective as the 100% commercialized solasodine. Therefore, *S. nigrum Linn.* extract can be used as an organic antibacterial substitute to the commercialized antibacterial drugs in treating *E.coli* 1057:H7 infections. Table 3 shows the summary of the statistical figures from AVOVA and post-hoc analysis.

Table 3. Comparative analysis of the significance of difference among set-ups and trials.

| **ANOVA** | **p-value (α=0.05)** | **Significance** | **Post-hoc analysis* (*Bonferroni* correction)** |
|---|---|---|---|
| Comparative analysis among set-ups | 0.000 | Significant | Difference is significant at p=0.016 between negative control set-up in comparison to other set-ups. |
| Comparative analysis among trials | 0.998 | Not significant | - |

*p=0.016 for three events per comparative analysis

Another set of ANOVA test was conducted to compare the results among trials. The test showed statistical no difference ($p>0.05$) in the measure of zone of inhibitions. This means that the consistency of the data from all trials did not change, and were not affected by any extraneous

 

variances which could possibly alter the result of testing. These findings were relevant in providing support to the effectiveness of isolated solasodine from *S. nirgum Linn*., and appropriateness of the methodology employed by the researcher. No post-hoc analysis was conducted since data revealed similar results across trials.

**Recommendations for Future Study**

Venture other phenotypic screening and isolation techniques of solasodine to isolate the pure phytochemical constituent against *E.coli* 0157:H7. Future researchers may also combine literature—proven phenotypic screening of solasodine against the bacteria. To fully accredit the result of the experimental set-up on the effectiveness of isolated solasodine, it is recommended to conduct phenotypic screening and antibacterial testing of the 3% phytochemical constituents of the 97% ± 0.18% isolated concentration of solasodine. The experiment was conducted *in vitro* application. It is recommended to test the isolated solasodine in vivo to determine its effect in other tissues. Therefore, side effects particularly on the study of its toxicity levels must also be considered in a future study.

## V. CONCLUSION

The virulent strain of *E.coli* 0157:H7 can cause serious health condition like bloody diarrhea and severe abdominal pain which is not characterized by most of its *E.coli* spp. Though there were no known therapy to treat its infections in the body and synthetic antibiotics are not recommended treatments, the search for other alternatives has boosted the literature body on attempting to use phytochemicals. The study has proven that solasodine can act as an antibacterial agent against the bacteria, hence the phytochemical can alleviate the *E.coli*-related infections. The phenotipically screened solasodine evaluated through in vitro disc diffusion assay was proven to be as effective as the commercialized solasodine. Therefore, the use of phytochemical solasodine from *S. nigrum Linn*. can hinder and inhibit bacterial growth.

It was concluded that the use of *S. nigrum Linn.* extract is a good organic alternative, in adequate quantity, to treat *E.coli*-related infections.

## ACKNOWLEDGMENT

The researcher acknowledges Mr. Richard Octavo for the technical assistance provided in the conduct of the study. He is also grateful to Mr. Rex Mercado for the recommendations of supplies, materials and guidance throughout the entire research endeavor.

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