Susceptibility of *Escherichia coli*, *Salmonella typhi* and *Shigella* spp to ethanoic extract of *Lawsonia inermis*

Original Research Article

ABSTRACT

Background: The acceptance of traditional medicine as an alternative form of healthcare and the development of microbial resistance to the available antibiotics has lead researchers to investigate the antimicrobial activity of plant extracts. **Aim:** This was to evaluate the antibacterial activity and potential effect of *Lawsonia inermis* leaves against three tests organisms namely: *Escherichia coli*, *Salmonella typhi*, and *Shigella*. **Methodology:** The *Lawsonia inermis* leaves powder was obtained from Nupur Company that produces the powder for hair dye (a100% henna). The extracts were boiled, macerated, soaked and ethanoic extracts of *Lawsonia inermis* was obtained and the implementation of the extracts to determine the antimicrobial activities onto culture was performed by the agar well diffusion method. Gentamicin, Ciprofloxacin and Cefataxine were used as control abtibiotics for the test organisms respectively. **Results:** The inhibition of each test organism was achieved in one or two extracts. *Escherichia coli* had the highest inhibition zone (7.25mm) from soaked extract with lowest inhibition zone (5.00mm) from boiled extract, *Salmonella typhi* had the highest inhibition zone (11.63mm) from boiled extract with lowest inhibition zone (8.25mm) from macerated extract, and *Shigella* had the highest inhibition zone of 19.50mm from soaked extract, and had the lowest zone of inhibition 12.63mm from boiled extract. Furthermore, the soaked ethanoic extract had inhibition zone ranging from 7.25mm-19.50mm. Also, the ethanoic extract boiled had inhibition zones ranging from 5.00mm – 12.63mm, and the ethanoic extract macerated had inhibition zone range of 6.63mm- 17.75mm. The inhibition zones produced by the controls drugs ranges from 25.00mm – 26.00mm (gentamicin), 20.00mm 22.00mm (ciprofloxacin), and 18.00mm – 21.00mm (cefataxime). The Statistical analysis was applied to the result using the one way ANOVA test to compare the differences in the means. **Conclusion:** The results indicated that there was no significant difference in the effects of the ethanoic extracts of Lawsonia inermis on the tests organisms *S. typhi*, *E. coli* and *Shigella* and the control drugs($p<0.05$, $F_{Cal} = 0.103$, $F_{Tab} = 4.257$).

Key words: *Lawsonia inermis,* traditional medicine, medicinal plants, antibacterial activity, *Escherichia coli*, *Salmonella typhi*, and *Shigella*

INTRODUCTION

The increase in prevalence of multiple drug resistance has slowed down the development of new synthetic antimicrobial drugs, and has necessitated the search for new antimicrobials from alternative sources[(1)]. The acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has lead researchers to investigate the antimicrobial activity of plant extracts. Medicinal plants are part and parcel of human society to combat diseases, from the dawn of civilization.

In traditional Indian medicine and the world, there exist a plethora of knowledge, information and benefits of herbal drugs or medicines where there is a great demand both in the developed and developing countries for primary healthcare because of their wide biological and medicinal activities with higher safety margins and lesser costs[(2)]. According to the World Health Organization (WHO), 2003 about 80 % of the population of developing countries being unable to afford pharmaceutical drugs rely on traditional medicines, mainly plant based, to sustain their primary health care needs[(3)].

About 20% of plants found in the world had been submitted to pharmaceutical or biological tests and a sustainable number of new antibiotics introduced in the market were obtained from natural or semi-synthetic resources[(4)]

In recent times, researchers focused on the use of herbal medicine are based on the premise that plants contain natural substances that can promote health, alleviate illness and improved quality of life. Today, we are witnessing a great deal of public interest in the use of herbal remedies, however, the healing activity may be slow with the use of plant extracts but have permanent cure against various diseases.

Though, the traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries and the need to discover new molecular structures as lead compounds[5, 6, 7].

Microbes have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. Though pharmacological industries are producing a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased to great extant to eradicate microbes with appropriate and efficient antimicrobial drugs to the patient more intensively with studies for natural therapies[8,9].

The problem of microbial resistance is growing and the attitude for the use of antimicrobial drugs in the future is still uncertain. Hence, measures must be taken to reduce this problem[10].

According to WHO medicinal plants are the greatest source to obtain a variety of drugs. The phytocompounds are the natural products and can be used in the control and cure of diseases such as Diabetes, Alzheimer, Parkinson, Arteriosclerosis etc. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency[11].

In rural areas of the developing countries, medicinal plants continue to be used as the primary source of medicine as traditional treatment for numerous human diseases[12]. It is estimated by the World Health Organization that approximately 75-80% of the world's population use plant medicines either in part or entirely[13]. According to Kim (2005)[14] there are approximately 500,000 plant species occurring worldwide, of which only 1% diseases causing bacteria.

Lawsonia inermis: Henna Lawsonia inermis, belongs to lythraceae also known as the loosestrife family. Henna is cultivated by many farmers for cosmetic and pharmaceutical purposes, it belongs to the group of plants that are popular in nature and all parts of the plant (root, stem, leaf, flower pod and seed) are of great medicinal important have been cultivated for traditional medicine because of its cosmetic agent and pharmacological activities worldwide [15]. Most importantly, the leave part of the plant contained a coloring compound called Lawson which is a red orange dye molecule, also known as hennotannic acid used for dyeing the skin, hair and fingernails as well as fabricks including silk, wool and leather[16]. Apart from the dyeing properties, the *L. inermis*, normally known as *hina*, or the henna tree in English, *Mehandi* in Hindi, *Shudi* in Bengali, *Goranta* in Kannada, *Mailanschi* in Malyalam, *Padchi-methi* in Marathi, *Dvivranta* in Sanskrit, *Gorata* in Telgu, *Aiyanam* in Tamil, *Monjathi* in Oriya, *Maduyanta* in Tibetan, the mignonette tree, and the Egyptian privet have been reported to have analgesic, hypoglycemic, hepato-protective, immune-stimulant, anti-inflammatory, antibacterial/fungal, antiviral and antiparasitic properties for the cure of renal lithiases, jaundice, wound healing and prevention of skin inflammation and leprosy. The species is named after the Scottish physician Isaac Lawson, a good friend of Linnaeus[7, 17].

(a. Source; Sarita *et al*., 2020)[19]

(b. Source; Jeba *et al*., 2019)[20]

Figure 1a-b): Pictograph of Henna, (*Lawsonia inermis*)

Phytochemical components: Phytochemicals are chemical compounds that naturally occurring in the plant parts such as flower, buds, fruits, barks, leaves, vegetables and roots to have defense mechanism and protect the plants from various diseases. They are also found in spices, and medicinal plants; and work in conjunction with other plant components as defensive mechanisms for the plants against

diseases and many external attacks. In addition to protective effects, they contribute to the plant's color, aroma and flavor. Phytochemicals are found to be accumulated in different parts of the plants, such as in the roots, stems and leaves[20, 21]. In humans, many phytochemicals have been found to be protective and preventive against many degenerative diseases and pathological processes such as in ageing, neurodegenerative disorder, atherosclerosis and inflammation[22]. Phytochemicals are categorized as primary and secondary constituents in which proteins, chlorophyll and common sugars are primary constituents while terpenoid, alkaloids, phenolic compounds are grouped into secondary constituents with various important pharmacological activities in Terpenoids, anaesthetic agents in Alaloids[20, 23, 24]. The phytochemical analysis of the aqueous extract of *Lawsonia inermis* is largely studied by many practitioners of traditional herbal medicines revealed the presence of carbohydrates, phenolic compounds, flavonoids, saponins, proteins, alkaloids, terpenoids, quinones, coumarins, xanthones, 6% fat, 2-3% resin and 7-8% tannins. *Lawsonia inermis* contained 2-hydroxy-1,4-naphthoquinone (lawsone). HPLC analysis showed that the extracts of *Lawsonia inermis* flowers, leaves and branches contained 116.9, 486.2 and 5.4 µg/g lawsone[25]. Polyphenols (equivalent to gallic acid), tannins (equivalent to catechin), flavonoids (equivalent to quercetin) and anthocyanins (equivalent to cyanindin), Other napthoquinone derivatives: 1,3-dihydroxy naphthalene, 1,4-napthaquinone, 1,2-dihydroxy-4-glucosylnaphthalene and 1,2,4-trihydroxynaphthalene-2-O-β-D-glucopyranoside were also isolated from the leaves of *Lawsonia inermis*[26, 27]. Henna is a whole glycosidase, able to break down the glycosidic bond when drawn in contact with hot water. Therefore lawsone has been extracted by maceration, infusion and digestion[28]. The main colouring agent of henna is lawsone (2-hydroxy- 1,4 naphthoquinone) which is particularly undiffused in the leaves foliage. The dry powder leaves of henna consist of 0.5 — 1.5% lawsone. Besides lawsone, the plant also contains esculetin, Gallic acid, hennadiol, betulinic acid, hennatannic acid coumarin, laxanthone, etc [19, 29, 30].

Antimicrobial Activity

The treatment of diseases inhibitory chemicals employed to kill micro-organisms or prevent their growth, are called antimicrobial agents. These are classified according to their application and spectrum of activity, as germicides that kill micro-organisms, whereas micro-biostatic agents inhibit the growth of pathogens and enable the leucocytes and other defense mechanism of the host to cope up with static invaders. The germicides may exhibit selective toxicity depending on their spectrum of activity. They may act as viricides (killing viruses), bacteriocides (killing bacteria), algicides (killing algae) or fungicides (killing fungi)[31].

Nature has been a source of medicinal agents, including all types of living organisms, for thousands of years with various energy sources of phytocompounds showing good action in controlling microbes[8, 32]. Many phytological studies have been conducted in Brazil and India[33]. The study of antimicrobial activity as well as cell toxicity of extracts from thirty plant species against five bacteria species and two fungi species was studied by Bhavani and Ballow (2000). It was concluded that ethanol extracts from 70 % of the plants were toxic to cell and only one of the species of Combretum duarteanum showed antimicrobial activity[8]. The antimicrobial properties of plants have been investigated by a number of researchers worldwide, especially in Latin America. In Argentina, a research tested 122 known plant species used for therapeutic treatments[33]. There has been a revival of interest in herbal medicines, to control major diseases and the need to discover new molecular structures as lead compounds. The healing activity may be slow with the use of plant extracts but have permanent cure against various diseases[6].

The antibacterial agents are classified in three categories: antibiotics and chemically synthesized chemotherapeutic agents, Non-antibiotic chemotherapeutic agents (Disinfectants, antiseptics and preservatives) and Immunological products. These drugs interfere chemically with the synthesis of function of vital components of microorganisms where the cellular structure and functions of eukaryotic cells of the human body provides selective toxicity of chemotherapeutic agents against bacteria. These differences provide us with selective toxicity of chemotherapeutic agents against bacteria in which these agents exhibit their antimicrobial activity through: cell-wall synthesis, protein synthesis, nucleic acid synthesis, enzymatic activity, folate metabolism or damage cytoplasmic membrane[31].

Yemeni traditional healer's uses ethanol extracts of 20 plants species for the treatment of pathogenic diseases. Both gram positive and gram negative bacteria used for the antibacterial screening of different plant species. Among all the plant species tested, L. inermis ethyl acetate extract was showed highest antibacterial activity[26]. Dama *et al*., (1999)[34] studied quinonic compounds from L. inermis in-vitro for antimicrobial properties. Kirkland and Marzin (2003)[35] conducted genotoxic studies on lawsone and suggested that it is a weak bacterial mutagen for Salmonella typhimurium strain TA98 and was more

clearly mutagenic for strain TA2637. Overall, it is suggested that L. inermis possess no genotoxic risk to the consumer. Antibacterial effect was also reported by the aqueous extract of leaves of L. inermis[36]. Aqueous, methanol and chloroform crude extracts of L. inermis leaves showed the in-vitro antimicrobial activity by inhibiting the growth of different strains of pathogenic bacteria[37].

For over five decades the inhibitory action of henna was shown against both gram negative and gram positive microorganisms with greater inhibitory action against *B. anthracis* as it stood out from tested bacteria[38]. Lawsone, the antimicrobial agent in henna exerted inhibitory effects upon common nosocomial urinary tract pathogen such as *E. coli*, *Proteus mirabilis*, *Klebsiella pneumonia*, *Pseudomonas aeroginosa*, and *Staphylococcus aureus* at certain concentration [39]

Salmonella typhi: Firstly, *Salmonella* infection of man and animals continues to be a distressing health problem worldwide. Far from disappearing, the incidence in developing countries has risen higher than expected. *Salmonella* are widely dispersed in nature, including the gastrointestinal tracts of domesticated and wild mammals, reptiles, birds, and insects with over 2,500 serotypes as defined by the somatic and flagellar antigens. Some *Salmonella* serotypes, such as *typhi* and *paratyphi* are highly adapted to humans and have no other known natural hosts. Others, such as *typhimurium* and *enteritidis*, have a broad host range and can infect a wide variety of animal hosts. These non-typhoid *Salmonella* can cause protean manifestations in humans, including acute gastroenteritis, bacteremia, and extraintestinal localized infections involving many organs. The widespread distribution of *Salmonella* in the environment, their increasing prevalence in the global food chain, and their virulence and adaptability result in an enormous medical, public health, and economic impact worldwide[40]. *Salmonella typhi* is a gram-negative enteric bacillus belongs to the family *Enterobacteriaceae*, a motile, facultative anaerobe that is susceptible to various antibiotics. According to World Health Organization (WHO), typhoid fever can be an issue in areas with overcrowding and poor hygiene where transmission is oral-fecal usually by ingestion of contaminated food and/or water[41]. Generally the incubation period ranges between 3 to 60 days with an average of 8 to 14 days. This period depends on the quantity of inoculum and host factors The clinical presentation of typhoid fever can be nonspecific ranging from mild symptoms (such as low grade fever, malaise, headache, and dry cough), to severe abdominal pain, intestinal perforation and neurologic manifestations. It can therefore resemble other diseases that are common in areas where typhoid fever is endemic, such as malaria, pneumonia, and tuberculosis and treatment consists of antibiotics, mostly fluoroquinolones or cephalosporins (according to antibiotic susceptibility), antipyretics, and individualized supportive therapy based on the patient's presentation[42].

Escherichia coli: Escherichia coli is a gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus Escherichia that is commonly found in the lower intestine of warm-blooded organisms (endotherms)[43]. *E. coli* is a type of bacteria that normally lives in human intestines and in the gut of some animals which can cause diarrhea as a result of eating contaminated food or water while many associate it with food poisoning. However, some types of *E. coli*, particularly *E. coli* O157:H7 can cause intestinal infection. In fact, 75% to 95% of urinary tract infections are caused by *E. coli*. *E. coli* is a normal resident of the bowel, which is how it makes it way to the urinary tract[44, 45]. Most *E. coli* strains do not cause disease, naturally living in the gut, but virulent strains can cause gastroenteritis, urinary tract infections, neonatal meningitis, hemorrhagic colitis, and Crohn's disease. Common signs and symptoms include severe abdominal cramps, diarrhea, hemorrhagic colitis, vomiting, and sometimes fever. In rare cases, virulent strains are also responsible for bowel necrosis (tissue death) and perforation without progressing to hemolytic-uremic syndrome, peritonitis, mastitis, sepsis, and gram-negative pneumonia; however, healthy individuals of all ages are at risk to the severe consequences that may arise as a result of being infected with *E. coli* and the rate and severity of infections are higher among children under the age of five, including as many as 380,000 deaths annually[46, 47]. The mainstay of treatment is the assessment of dehydration and replacement of fluid and electrolytes. Administration of antibiotics has been shown to shorten the course of illness and duration of excretion of enterotoxigenic. The antibiotic used depends upon susceptibility patterns in the particular geographical region. Currently, the antibiotics of choice are fluoroquinolones or azithromycin, with an emerging role for rifaximin[48, 49].

Shigella: Shigellosis is a bacterial infection that affects the digestive system which is spread through contaminated water and food or through contact with contaminated feces. The bacteria release toxins that irritate the intestines and cause an infection known as shigellosis with th primary symptom of diarrhea, fever, and stomach cramps usually begin 1–2 days after infection and last 7 days. Most people recover without needing antibiotics, however, people with severe illness and those with underlying conditions that weaken the immune system are given antibiotics to shorten the duration of illness (by about 2 days) and

might help reduce the spread of infection to others. Personal hygiene measures can help protect one from the infection. Shigella is a genus of bacteria that is Gram-negative, facultative anaerobic, non-spore-forming, nonmotile, rod-shaped and closely related to *E. coli*. The genus is named after Kiyoshi Shiga, who first discovered it in 1897 with natural habitat in humans and gorillas and causes diseases in primates[50, 51]. Shigella is one of the leading bacterial causes of diarrhea worldwide, causing an estimated 80–165 million cases. The number of deaths it causes each year is estimated at between 74,000 and 600,000[52, 53]. Contacting the healthcare provider or facility is a major way of control, drinking plenty of fluids to prevent dehydration, ciprofloxacin and azithromycin are recommended as oral antibiotics can shorten the time of fever and diarrhea by aout 2 days[54].

Statement of Problem

The complexity of available drugs, the resistances of microorganisms and finances to treat diseases in developing countries especially in resource limited settings is been on the rise due to the increase in standard of living and increase in the cost of health care to tackle diseases and the organisms causing these diseases. Lack of information to individuals living in areas with available plants with antimicrobial properties is a principal challenge. Thus, the antimicrobial action of the plant extracts of *Lawsonia inermis* (Henna) against *Salmonella typhi, Shigella spp.,* and *Escherichia coli* will confine for the resolution and treatment of these infectious microorganisms while the enlightenment of the individuals in these localities will be achieved. The study therefore, aimed to ascertain and investigate the antimicrobial activity of *Lawsonia inermis* (Henna) leave extract against *Salmonella typhi, Shigella spp.,* and *Escherichia coli*.

Significance of Study

Investigating the antimicrobial activity of *Lawsonia inermis* extracts on *Salmonella typhi, Shigella,* and *Escherichia coli* as infections and diseases of medical importance in humans, will aid in the treatment of such infections and diseases in the simplest, effective and cost effective extracts of the plant. About 80% of developing countries, citizens used traditional medicine based on plant products[55]. Thus, this study will improve the standard of living of individuals living in developing countries where the plant species are available. Also, the availability of *Lawsonia inermis* will add to the pool of knowledge to the resource limiting settings and in the identification of naturally antimicrobials which may be potentially used in the production of new clinical drugs by the pharmaceutical industries.

MATERIALS AND METHOD

Study Area: The study was carried out at the College of Research and Technology, Shaheed Udham Singh Group of Institutions. The college is located in Tangori town, Mohali region, Punjab. The region has 4 weather seasons, winter, spring, summer and fall. The university covers a landmass of 100 square meters and is located at latitude 30.589055, longitude 76.704130, it is found 11 miles away from Chandigarh the state capital of Punjabi.

Sample Collection: Henna leaves; The *Lawsonia inermis* leaves powder was obtained from Nupur Company that produces the powder for hair dye (a100% henna). Microorganism; the test microorganisms were obtained from sewage water from the environment. The target organisms, *E. coli S. typhi and Shigella* were obtained by performing serial dilution, culture streaking for pure isolation on nutrient broth and agar respectively.

Preparation of Extracts

Macerate: The Henna powder used was measured (150g) by inserting it in a container with a solvent (50ml Ethanol) and allowed to stand at room temperature for a period of minimum 3 days with frequent agitation. The mixture was thereafter filtered using the Whatman's No 1 filter paper[56].

Soaking: The extract powder was measured (50g), soaked in 150 ml of solvent (Ethanol) in a 500 ml sterile conical flask and covered with cotton wool. It was then plugged and wrapped with aluminum foil and vigorously shaken. The mixture was left to stand overnight (24 h) in a shaking water bath at 40°C. The mixture was then filtered using a Whatman's No. 1 filter paper[57].

Boiling: The Henna powder measured (50g), meshed and placed in a container containing 150ml of Ethanol (solvent), boiled for 30minutes with constant stirring, allowed to cool and it was filtered[58].

Preparation of Agar: The agar was measured according to the required quantity of water and producers instructions. The agar mixture is sterilized in a flask in the autoclave at 121°C for 15 min at 15 pounds per

square inch (psi) and cooled to 40 - 50°C was poured aseptically, in the volume of 20ml each, into sterilized Petri dishes and allowed to harden under room temperature. The plates were placed in the incubator for 24h at 37°C to check for its sterility prior to antimicrobial test[59].

Preparation of McFarland Standard: The turbidity standard equivalent to 0.5 McFarland standards was prepared by adding 1ml of concentrated tetraoxosulphate (VI) acid to 99ml of water. 0.5g of dehydrate barium chloride was dissolved in 50ml of distilled water. After which 0.6ml of the barium chloride solution was added to 99.4ml of the acid solution. The mixture was mixed properly and a small portion of the turbid solution was transferred into a corked tube and stored properly at room temperature ready for use[59].

Determination of Antimicrobial Activity: The test organisms were each streaked and spread on the surface of the prepared nutrient agar medium. The inoculated plates were allowed for 30min at room temperature for the organisms to pre-diffuse. After which, the inoculated plates were punched with a 5mm cork borer to make wells in the agar plate. The wells were each filled with the different plant extracts aseptically and the plates were incubated at 37°C for 24h. The antibacterial activity of the active constituents of the plant extracts were tested on each of the test organisms and were determined by measuring inhibition zone diameter (IZD) in millimeters (mm) [59].

Data Analysis: The results obtained from the antibacterial assay were analyzed using descriptive statistics and one way Analysis of variance (ANOVA) tests. The experimental design used was completely randomized design.

RESULTS AND DISCUSSION

Amos *et al*., in 2016 and Rayavarapu *et al*., in 2011 reported a significant evaluation of plant materials to provide a major source of natural therapeutic remedies used for antimicrobial properties against aqua pathogenic bacteria and fungi[59,8]. In this study, the antimicrobial activity of ethanoic extract of *Lawsonia inermis* was verified against *S. typhi, E. coli* and *Shigella*.

In table 1, the inhibition zones upon the application of soaked ethanoic extracts revealed the highest inhibition zone of 19.50mm in *Shigella spp* isolated culture. The lowest inhibition zone was at 5.00mm of the isolate *E. coli* upon the application of the Boiled ethanoic extract, and of the isolate *Shigella* with the application of Boiled ethanoic extract.

In table 2, the mean zones of inhibition (mm) observed upon the introduction of ethanoic extracts of *Lawsonia inermis* to bacterial isolates shows the highest mean of inhibition zone obtained was 19.5mm from application of soaked ethanoic extracts of *Lawsonia inermis* to *Shigella*. The lowest inhibition zone observed was 5.00mm of produced to *E. coli*.

The result achieved from Tables 1 and 2 shows that the soaked ethanoic extract inhibited the growth of test organisms *S. typhi, E. coli* and *Shigella*, with *S. typhi* and *Shigella* having a high susceptibility than *E. coli* which has a lower susceptibility range. The macerated ethanoic extract had a low susceptibility effect against *S. typhi* and *E. coli* with a medium to high sensitivity towards the isolate *Shigella*. The boiled ethanoic extract had no inhibitory effect against *E. coli* with a medium inhibitory effect against *S. typhi* and *Shigella* isolates. Maceration at an elevated temperature (50℃) increases the extraction efficiency and have a stronger antimicrobial effect. The plant leaves powder of *Lawsonia inermis* is shown to have varying active components that are release into the solvents through various methods of extraction. Therefore for its use against certain confirmed organisms, a particular method of extraction is employed to achieve a better antimicrobial effect.

In table 3, the mean zones of inhibition of bacterial isolates upon the introduction of control antibiotics (Gentamicin, Ciprofloxacin and Cefataxime) used for *Shigella spp, E. coli* and *S. typhi* respectively. The highest mean zone of inhibition of antibiotics is 26.00mm observed from Gentamicin upon its application on *Shigella.* The lowest mean zone of inhibition is 18.00mm obtained from Cefataxime upon its introduction on *Salmonella.*

The result from table 3 indicated that the zones of inhibition for each of the controls used in the antibacterial assay had Ciprofloxacin producing inhibitory zone ranging from 20-22 mm, Cefataxime produced zones of inhibition ranging from 18-20 mm, and Gentamicin produced zones of inhibition ranging from 25-26mm. Cefataxime shows to have the lowest zone of inhibition against *S. typhi*, while Gentamicin had the highest zone of inhibition against *Shigella*. The table indicates that the test organisms are sensitive to the antibiotics used against them as controls. Using the analysis ANOVA, the data supports that there is no difference in the ranges of effect of antibiotics against each of the organisms (at $p<0.05$, F Cal = 0.208, F Tab = 5.1433; at $p<0.01$, F Tab =10.925). At both confidences, F Cal is less than F tab and therefore the H_o is accepted.

In the result section (Table 4), it was shown that there was no significant difference between the zones of inhibition produced by the controls and Ethanoic extract of *Lawsonia inermis* (at $p<0.05$, $F_{Cal} = 4.201$, $F_{Tab} = 4.066$) and thus indicating that the usage of ethanoic extracts of *Lawsonia inermis* has a relatively close effect to the antibiotics used as control. This indicates that the ethanoic extracts have a probability of providing similar effects against target microorganisms at $p<0.01$, $F_{Cal} = 4.201$, $F_{Tab} = 7.591$ when used as treatment. However, a longer period of usage of the ethanoic extracts may be required to obtain a desired effect of inhibition on the target organisms.

This study has indicated that the extraction of the active components of *Lawsonia inermis* through ethanoic extraction has improved the release of active compound into the solvent and provides a better concentration of the active compounds to produce inhibitory effects against targeted microorganisms. It is shown that the different extraction methods obtained a better concentration of active compounds which shows a medium inhibitory effect against the targeted microorganisms.
The Statistical analysis were applied to the result Tables 2,3 and 4 using the one way ANOVA test to compare the differences in the means indicated that there was no significant difference in the effects of the ethanoic extracts of *Lawsonia inermis* on the tests organisms *S. typhi, E. coli* and *Shigella*. ($p<0.05$, F Cal = 0.103, F Tab = 4.257) as compared with the control drugs.

Table 1: Zones of Inhibition (mm) on Cultures of *S. typhi*, *E. coli* and *Shigella* upon the application of ethanoic extracts of *Lawsonia inermis*

TEST ORGANISMS	EXTRACTS					
	SOAKED (mm)*		MACERATED (mm)		BOILED (mm)	
Escherichia. coli	8.25	6.25	7.75	5.5	5	5
Salmonella. typhi	10.5	8.5	7.75	8.75	11	12.25
Shigella	19.5	19.5	16	15.5	5	20.25

*millimeters (mm)

Table 2: Mean Zones of Inhibition (mm) on the Cultures of *S. typhi*, *E. coli* and *Shigella* upon the application of ethanoic extracts of *Lawsonia inermis*

TEST ORGANISMS	EXTRACT		
	SOAKED (mm)	MACERATED (mm)*	BOILED (mm)
Escherichia. Coli	7.25	6.63	5
Salmonella typhi	9.5	8.25	11.63
Shigella	19.5	15.75	12.63

*millimeters (mm)

Table 3: Mean Zones of Inhibition (mm) on cultures of *S. typhi, E. coli* and *Shigella* upon the application of control antibiotics

TEST	EXTRACT

MICROORGANISM	ANTIBIOTICS	SOAKED (mm)	MACERATED (mm)	BOILED (mm)*
Escherichia. Coli	Ciprofloxacin	20	22	21
Salmonella typhi	Cefataxime	19	21	18
Shigella	Gentamicin	25	26	26

*millimeters (mm)

Table 4: Mean Zones of Inhibition (mm) on cultures of *S. typhi, E. coli* and *Shigella* upon the application of ethanoic extracts of *Lawsonia inermis* and control antibiotics

TEST ORGANISMS	EXTRACTS AND ANTIBIOTICS			
	SOAKED (mm)	MACERATED (mm)	BOILED (mm)	ANTIBIOTICS (mm)*
Escherichia. coli	7.25	6.63	5	21
Salmonella typhi	9.5	8.25	11.63	19.3
Shigella	19.5	15.75	12.63	25.7

*millimeters (mm)

CONCLUSION

The resulting effect of this study paved a way in the antimicrobial potential of *Lawsonia inermis* ethanoic extracts against *S. typhi, E. coli* and *Shigella* which indicated that the extracts of *Lawsonia inermis* has active compounds that have antimicrobial effect against the test bacteria and can be useful in their treatment.

RECOMMENDATION

The discovery of new novel drugs enhances the connection between traditional medical practitioner's reports and modern medicine reports to develop more active, stable and efficient drugs as suggestions to the unlimited potential of *Lawsonia inermis* plant. However, this ancient concept should be carefully evaluated in the light of modern medical practices and can be utilized fully or partially if found suitable.

Based on the results obtained from this research, the following recommendations are made

1. The ethanoic extracts are to be explored from its effect against other microorganisms and human consumption.
2. The development of efficient extraction method is to be acquired to obtain or exploits the plants potential rather than the traditional methods of extraction and
3. The development of better chemicals or solvents to harvest the desired active compounds should be explored.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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