PHYTOCHEMICAL ANALYSIS AND POTENTIAL INHIBITORY PROPERTY OF TUBLI (*Derris elliptica* Benth) ROOT EXTRACT ON *Escherichia coli* AND *Staphylococcus aureus*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

ABSTRACT

Medicinal plants contain bioactive compounds which are used for thetreatment of various human diseases. One of these plants is the Tubli(*Derris elliptica* Benth) which comes from the genus of the familyPapilionaceae locally known in Southeast Asian Countries as "Derris" or Tuba" and "Tubli" or "Tugling-pula" in Philippines. In this paper, is reported inhibitory property of this plant which hadbeen studied against *Escherichia coli* and *Staphylococcus aureus* to find new substances with antimicrobial properties. The main purpose of the study was to identify the biochemical compounds of Tubli (*Derris elliptica* Benth) that inhibit the growth of *Escherichia coli* and *Staphylococcus aureus*; todetermine the result of the mean average of zone inhibition between *Escherichia coli* and *Staphylococcus aureus* and todifferentiate the mean average of zone of inhibition between *Escherichia coli* and *Staphylococcus aureus*. Theexperiments conducted used the Completely Randomized Designed (CRD). The data were analyzed using T-Test.Phytochemical analysis of Tubli (*D. elliptica* Benth) revealed the presence of tannins, flavonoids, alkaloids, saponins, and steroids which contributed to the antimicrobial ability of the root extract. It was found out that Tubli (*D. elliptica* Benth) was more active against the *S. aureus* having the total average zone of inhibition of 24.66 mm than the *E. coli* having the total average zone of inhibition of 20 mm.T-Test revealed that there is a significant difference on zone of inhibitions of the *S. aureus* and *E. coli* tested under Tubli (*D. elliptica* Benth) ethanolic root extract. The presence of phytochemicals suggests that *D. elliptica* has a potential antimicrobial property that could serve as a natural source of traditional medicine for treatment of various diseases.

Keywords: Phytochemical Analysis, Inhibitory Property, Tubli, Escherichia coli, and Staphylococcus aureus.

1. INTRODUCTION

1.1 Background of the Study

"Humankind has provided many things by nature over the years, including the tools for the first steps at therapeutic intervention. Early civilization relied on plant extracts used as medicines. As of now, plant materials arestill important resources for combating ailments, such as contagious diseases and many of herbal plants are studied for the discovery of new medicines, preservatives, industrial chemicals, and agrochemicals" (Habila, et al., 2011).

A medicinal plant has phytochemicals that areused for traditional medicine and used as predecessor for chemo-pharmaceutical semi synthesis (Doughari, et al., 2009). The leaves, fruits, and roots of herbal plants are useful for treating human diseases since it contains phytochemicals that have medicinal properties (Doughari, et al., 2009; Wadood, et al., 2013). These phytochemicals are responsible for protection frommicrobial and pest disturbance (Doughari and Obidah, 2008). Moreover, herbal extracts have been discovered to be safer medicines with minimum side effects when compared to chemical drugs (Poojary, et al., 2015).

Tubli (*Derris elliptica* Benth) is also considered as a herbal plant (Teng, 2008). This comes from the genus of the family Papilionaceae (Orwa, et al.,2009), and locally in SoutheastAsian Countries as "Derris" or "Tuba" and in Thailand as "Lotin" or "Hang laidaeng" (Gupta, 2007) and "Tubli" or "Tugling-pula" in Philippines (Teng,2008). Tubli is a climbing plant with branches covered with brown hairs and pinnate shape of leaves which measures 30 to 50 cm long. The lax of racemes has a measurement of 15-

30 cm in length and blossoms a reddish flower. The pods measure 5-8 cm long and contain seeds which are flat and reniform with brown or black color (Teng, 2008).

Tubli plant isoften confined to low altitudes but it can stay alive for months during summer and it is abundant in roadsides, forest edges, and along rivers in the tropical country up to 1500 m altitude(Orwa, et al., 2009). In the Philippines, it is widely spread in mountain ranges that have water system at a moderate temperature from the north of Luzon to Mindanao islands (Teng, 2008).

Throughout Southern Asia and the Pacific, *D. elliptica* pounded root is classified as one the strongest fish poison over the centuries. Recently, it was discovered that it could be an effective insecticide. It is also traditionally used to apply to the skin to prevent the development ofabscesses against itch, leprosy, andantisepsis. In Thailand, they used Tubli stem as a blood tonic and the roots as an emmenagogue (Orwa, et al., 2009).

Since tubli (*D. elliptica*) possesses medicinal value and insecticidal property, this study focused on the investigation of phytochemicals present in the root extract and assessed its antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus.*Thus, this study was conducted in the Department of Science and Technology-Caraga, CSU Campus, Ampayon, Butuan City during the first semester of Academic Year 2016-2017.

1.2Conceptual Framework of the Study

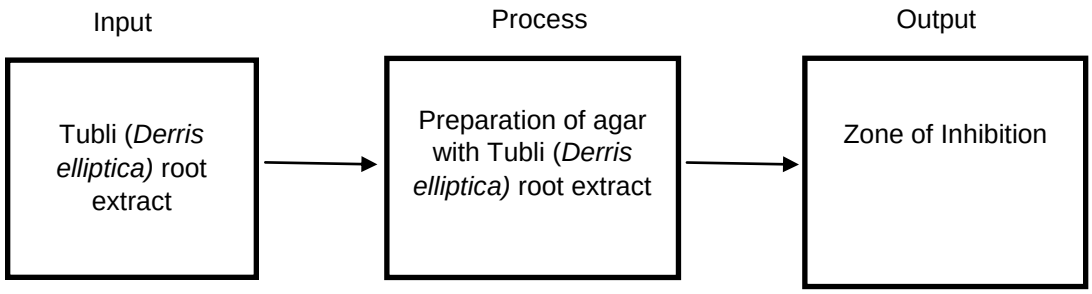

Figure 1. Figure 1 shows the research paradigm of the study, specifically the relationship between the input Tubli (*D. elliptica*) root extract, the process (Preparation of Agar media) with Tubli (*D.elliptica* root extract), and the output Result of Inhibitory Property evaluation.

1.3Statement of the Problem

The purpose of this study was to focus on the investigation of phytochemical content and determine the inhibitory property of Tubli (*D. elliptica*) root extract on *Escherichia coli* and *Staphylococcus aureus*.

Specifically, it attempted to answer the following questions:

1. What are the phytochemicals present in Tubli (*Derris elliptica*) root extract?
2.What is the mean average of zone of inhibition between*Escherichia coli* and *Staphylococcus aureus*?
3. Is there a significant difference in the mean average of zone of inhibition between *Escherichia* and *Staphylococcus aureus*?

2. LITERATURE REVIEW

Phytochemicals

Plants contain chemicals which serve as its secondary metabolites (Yao et al., 2004) these chemicals are not necessary nutrients but they can affect health (Temple, 2000). Flavonoids, isoflavones, flavones, phenolic acids, and glucosinolates are common types of phytochemicals. The formation of phytochemicals structure is due to the process of conjugation of sugar and aglycones (Luthria

&Natarajan, 2009). The study of natural products is called Phytochemistry. These Phytochemicals are present in vegetables, fruits,drinks and preservatives (Doughari&Obidah, 2008). In plants, phytochemicals serve as protection and defense mechanism of plants against pest and microbial organism (Krishnaiah et al., 2007).

In almost allplants, 10-15% of concentrations are alkaloids. They are water soluble and heterocyclic compound because they contain nitrogen in a negative oxidation state that can form salt with acids. Most of the alkaloids are having definite melting points, a crystalline solid, colorless, and bitter in taste. It activated and inhabited duringthe central process at cellular and organ level in animals. They impair the functioning in theliver and kidney. Some alkaloids affect thereproductive system of animals. Moreover, alkaloids possess allergic and antimitotic effects at cellular level. Alkaloids play a vital role in chemical ecological perspectives like plant-microbial interaction, plantherbivore interaction, and plant-plant interaction. Though poisonous, many alkaloids have a physiological effect that has medicinal value that can cure diseases including diabetics, cardiac dysfunction, cancer, malaria, etc. In addition, Alkaloids are also used as anesthesia to relieve pain (Mahajan, et. al., 2014).

"Flavonoids are another type of phytochemicals which consist of a large group of polyphenolic compounds having a benzo-y-pyrone structure and are ubiquitously present in plants. Plants can synthesize them in response to infectionwhich is hydroxylated phenolic substances. Flavonoids have potential pharmaceutical properties because of its antioxidant activities. Free radicals are scavenged by functional hydroxyl groups of alkaloids because it mediates their antioxidant effects. Due to its high antioxidant capacity both in vivo and in-vitro systems flavonoids have health-promoting properties. In addition, flavonoids have protective effects on infectious and degenerative diseases like cancers, cardiovascular diseases, and other age-related diseases" (Kumar and Pandey, 2013).

Saponins are structurally constructed of sugars and aglycone. In addition, saponins serve as a natural detergent. Saponins havecommercial and industrial applications ranging from their uses as a source of raw-materials for the manufacture of steroid hormones in the pharmacological industry, to their functions as food additives, photographic emulsions ingredients,fire extinguishers, and other industrial applications. Furthermore, they also exhibit different biological activities and have been investigated to create new natural medicines (Tamura, et. al., 2012).

Terpenoids are natural occurring compounds in which aremostly present in plants. They are volatile compounds which give the fragrance in plants and flowers. They usually occur in the fruits and leaves of higher plants, citrus, eucalyptus and conifers (Bano, 2007).Tannins are compounds which are displeasing but do not affect health which is obtained upon plants decomposition process. Tannins affect the sense of taste, odor of water and can make a yellow cast in water which is difficult to remove. Tannins are polyphenols that shrink, bind or precipitate proteins. In addition, tannins act as defiance system and protection of trees against pest and minute organisms. It is commercially used in tanning, refining beer and wine, dyeing, photographyand astringent in medicines (Ashok, 2012).

Further, rotenone is present in the Cube plant crude extracts that can poison fish and insect. It is isolated from tropical and subtropical members of pea family which grow in Southeast Asia and SouthAmerica. In addition, rotenone has a g-eat potential commercial value as insecticides and pesticides on food crops or fish poison (Ott, 2000). The leaves, roots, stem barks, flowers, or their combinations, essential oils of cube plants have been practices in the treating infection cause by pathogen and microbial diseases due to its diverse phytochemicals (Rios and Recio, 2005).

Economic Importance of Tubli

"Tubli (*Derris elliptica*) is a rambling climber, with branches covered with brown hairs and pinnate leaves which measure 30 to 50 cm long. The leaflets are usually oblong which range from 9 cmto 13 cm and when g-own matured the upper part measures up to 10-15 cm long. The lax of racemes can reach 15 to 30 centimeters in length and blossoms reddish flower. The pods reach up to 5 to 8 centimeters long and contain seeds which are flat and reniform with brown or black color" (Teng, 2008). When it reaches

18 months of age it begins to flower and fruit normally. After fertilization, its pods start to ripe for about 4 months. In cultivation, the fruiting of Derris is rare (Orwa, etal., 2009).

Morphology of *Escherichia coli*

"*Escherichia coli* is a Gram-negative rod (bacillus) in the family Enterobacteriaceae. Most *E. coli* are normal commensals found in the intestinal tract. Pathogenic strains of this organism are distinguished from normal flora by their possession of virulence factors such asadhesins, toxins, and biofilm formation. The specific virulence factors can be used, together with the type of disease, to separate these organisms into pathotypes" (Ateba and Bezuidenhout, 2008).

"Enterohemorrhagic*Escherichia coli* (EHEC) strains are a highly pathogenic subgroup of Shiga toxin—producing E. coli (STEC) that cause severe human diseases, including bloody diarrhea and hemolytic uremic syndrome (HUS)" (Karch, et al., 2005). "The ability to cause severe human disease differentiates EHEC from other STEC found in the environment that is less pathogenic or nonpathogenic. *E. coli*0157:H7 is the most frequent EHEC implicated as a cause of HUS but non—0157:H7 EHEC are variably present as the only pathogens in stools from HUS patients" (Karch, et al., 2005).

"EHECs are transmitted by the fecal—oral route. They can be spread between animals by direct contact or via water troughs, shared feed, contaminated pastures or other environmental sources. Birds and flies are potential vectors. In one experiment, EHEC 0157:H7 wastransmitted in aerosols when the distance between pigs was at least10 feet. The organism was thought to have become aerosolized during high pressure washing of pens, but normal feeding and rooting behavior may have also contributed" (Ateba and Bezuidenhout, 2008).

"The reservoir hosts and epidemiology may vary with the organism. Ruminants, particularly cattle and sheep, are the most important reservoir hosts for EHEC 0157:H7. A small proportion of the cattle in a herd can be responsible for shedding more than 95% of the organisms. These animals, which are called super-shedders, are colonized at the terminal rectum, and can remain infected much longer than other cattle. Super-shedders might also occur among sheep" (Alam and Zurek, 2006).

"*E. coli* can be killed by numerous disinfectants including 1% sodium hypochlorite, 70% ethanol, phenolic or iodine—based disinfectants, glutaraldehyde and formaldehyde. This organism can also be inactivated by moist heat (121°C [250°Fl for at least 15 min) or dry heat (160—170°C [320-338°Fl for at least 1 hour). Foods can be made safe by subjectingthem to a minimum temperature of 160°F/ 71°C. Ionizing radiation or chemical treatment with a sodium hypochlorite solution may reduce or eliminate bacteria on produce" (Seto, et al.,2007).

Morphology of *Staphylococcus aureus*

"*Staphylococcus aureus* is a gram-positive coccus pathogen. *S. aureus* is one of the main causes of hospital- and community-acquired infections which can result in serious consequences" (Diekema, et al., 2001). "Nosocomial *S. aureus* infections affect the bloodstream, skin, soft tissues and lower respiratory tracts.*S aureus* can be a cause of central venous catheter-associated bacteremia and ventilator- assisted pneumonia. It also causes serious deep-seated infections, such as endocarditis and osteomyelitis" (Schito, 2006). "In addition to the infections listed above, *S. aureus* is often responsible for toxin-mediated diseases, such as toxic shock syndrome, scalded skin syndrome and staphylococcal foodborne diseases (SFD). Hospitalized patients are particularly exposed to *S. aureus* infections due to their compromised immune system and frequent catheter insertions and injections" (Lindsay and Holden, 2004).

"Penicillin was used in order to combat this organism in the year 1940, and all isolates were sensitive to penicillin during that period. With this antibiotic, the mortality rate of bacteremia caused by aureus decreased from a striking 80% to 25%"(Ladhani, 2005). "While In 1944, the first penicillin-resistant strain of *S. aureus* was discovered. As clinicians used more andmore penicillin's as the primary treatment for *S. aureus* infection, the prevalence of penicillin-resistant strains increased in the 1960s. It was during this time that a new class of antibiotics was developed to target this pathogen specifically. This class of

antibiotics is known as the penicillinase-resistant penicillin's, which includes nafcillin, dicloxacillin, and oxacillin. One year after this class of antibiotic was developed, a resistant strain known as Methicillin-resistant *Staphylococcus aureus* (MRSA) was discovered in both the United States and the United Kingdom" (Zaoutis, 2006).

"Methicillin-resistant *Staphylococcus aureus* (MRSA) became increasingly prevalent, especially in larger hospitals, in the 1970s. From the late 1970s to the early 1990s, Methicillin-resistant*Staphylococcus aureus* (MRSA) was usually a hospital-acquired pathogen. It was not until 1998 that the first case of CAMRSA in children was identified, since then CA-MRSA has become more prevalent in children throughout the United States" (Zaoutis,.2006).

3. METHODOLOGY

3.1 Research Design

The experiments conducted used the Completely Randomized Designed (CRD)strategy. Completely randomized designs are the simplest in which the treatments are assigned to the experimental units completely at random. This allows every experimental unit, i.e., plot, animal, soil sample, etc., to have an equal probability of receiving a treatment. The test subjects are assigned to treatment levels of the primary factor at random. Levels of significance between treatments were measured using T-Test on CRD Experiment on inhibitory property of *Derris elliptica* on growth of *Staphylococcus aureus* and *Escherichia coli* were conducted usingCRD.

3.2 Treatments

Treatment 1 iscomposed ofthe agar growth media (*Staphylococcus aureus*) + *Derris elliptica* root extract. While the treatment 2 consists of the agar growth media (*Escherichia coli*) + *Derris elliptica* root extract. There were 3 replicates in each treatment.

3.3 Collection and drying of *Derris elliptica* roots

Roots of Tubli (*D. elliptica* Benth) were gathered and weighed253.41 g. The collected tubli roots were washed under running tap water and air dried at room temperature for 20 days.

3.4 Extraction of Fresh Roots Materials

The dried roots weighed 253.41 g in an Erlenmeyer flask and weretreated with sufficient 80% ethyl alcohol to completely submerge the material. The flask was then covered and the material was kept and soaked for 48 hours. After soaking, the roots were filtered through Buchner funnel with gentle suction. The flask and dried roots material were rinsed with fresh portions of alcohol. Then the dried roots and washing material were transferred to the funnel and the washings with the first filtrate. Gentle suctionwas applied to complete the collection of the roots extract and the roots residues were discarded. The filtrate was concentrated under the vacuo at 40°C. Then the extract volume of the concentrated extract was measured. Rotary evaporator machine was used to test and evaluate the phytochemicals present in the root extracts.

3.5 Preparation of the Test Organisms or Inoculum

The recommended set of assay organisms for the antimicrobial screening program were represented the major group of microbial flora: *Staphylococcus aureus* - gram positive cocci and *Escherichia coli*- gram-negative short rods. The 0.5 M McFarland standard was used to adjust the turbidity of the inoculums prior to the cotton swabbing of the agar plates for the antimicrobial assays. The standard was prepared and subjected to quality control prior to use. The standard contained approximately 1.5x 10 CFU/m1 of the test organism.

3.6Preparation of 0.5 McfarlandStandard

The 0.5 ml of 0.048 M BaC12 (1.175%w/vBaC12.2H20) was mixed to 99.5 ml of 0.36NH2S04(1%v/v). 5 ml was distributed into screw-cap tubes of the same dimension as those were used in preparing the culture suspension. The tubes were then tightly sealed and stored in the dark room temperature. Before used, the turbidity standard was shaken vigorously on a mechanical vortex mixer.

3.7 The Broth Culture

1. *Preparation of the nutrient broth*

Preparation of 1000ml solution was referred to label. A loop full of bacteria (g-am positive) was taken from the culture slant and inoculated in 500ml nutrient broth. The culture broth was then inoculated for 24hours at 350C and it was observed for turbidity which was the indicative of microbial growth.

2.*Adjusting the turbidity of the inoculum*

The adjusted turbidity served as the inoculums for the microbial assay and served as the inoculums which were swabbed onto agar plates.The 5 ml of the culture broth was aseptically transferred in sterile screw-capped tubes. The bacterial suspension was agitated on a vortex mixer and immediately compared against the 0.5Mcfarland standard preparations.

3.8 Preparation of Treatment Agar

A.*Staphylococcus aureus*

15m1 of melted nutrient agar was poured into dry and sterile petri dishes and they were allowed them to solidify. A sterile cotton swab wasmoistened into the *Staphylococcus aureus* (inoculum) suspension. The cotton swab with wooden applicator handles was used. Then asterile cotton swab was dipped into a suspension of the *Staphylococcus aureus* /inoculum. The moistened swab was then pressed and rotated firmly against the inside wall of the tube just above the fluid level to remove the excess liquid.

B.*Escherichia coli*

15ml of melted nutrient agar was poured into dry and sterile Petri dishes and allowed them to solidify. A sterile cotton swab was moistened into the *Escherichia coli* (inoculum) suspension. The cotton swab with wooden applicator handles was used. Then a sterile cotton swab was dipped into a suspension of the *Escherichia coli*/inoculum. The moistened swab was then pressed and rotated firmly against the inside wall of the tube just above the fluid level to remove the excess liquid.

3.9 Cotton Swabbing

A.*Staphylococcus aureus*

The *Staphylococcus aureus* was aseptically swabbed into a solidified nutrient agar by streaking the swab over the entire surface of the agar plate 3x, and rotated the plate 60 aftereach application to ensure an even distribution of the inoculum on the surface of the medium and the swabbed plates stand for 5minutes.

B.*Escherichia coli*

The *Escherichia coli* was aseptically swabbed into a solidified nutrient agar by streaking the swab over the entire surface of the agar plate 3x, and rotated the plate 60 degrees after each application to ensure an even distribution of the inoculum on the surface of the medium and the swabbed plates stand for 5 minutes.

3.10 Disposal of Laboratory Waste

Wastes that accumulated during the experiment were properly disposed according to standard laboratory waste disposal procedures.

3.11Data Gathering (Zone of Inhibition)

3.11.1The Plates

The "HALO" or "Clearing" was looked around the disc which was known as the zone of inhibition. The plates were inverted and using a ruler, the diameter of each inhibition zone was measured in millimeters and the result was expressed as mm diameter zone of inhibition. The diameter of the paper disc used in the assay wasrecorded in mm.

3.11.2Analyzing the Results

< 10 mm of the diameter of zone of inhibition may be expressed as in active, 10-13 mm means partially active, 14-19 mm is active while > 19 mm is very active.

3.11.3Statistical Treatment

Level of significance between treatments was measured usingT-Test on CRD Experiment on inhibitory property of *Derris elliptica* on growth of *Staphylococcus aureus* and *Escherichia coli.*

4. RESULTS AND DISCUSSIONS

Table 1 shows the ethanolic root extract of *Derris elliptica* Benth is a promising medicinal plant because of its variety of phytochemicals which were reported to have some key bioactivities. Tannins are very much astringent in nature and has high potential treating intestinal disorders such as diarrhea and dysentery (Akinpelu and Onakoya,2006). Its presence also aids in wound healing (Okwu and Josiah, 2006). *Derris elliptica* roots also prevent damage caused by free radicals in the body by neutralizing them. This can be explained by the presence of flavonoids in the plant, as flavonoids are known to act as antioxidant (Stauth, 2007). It also functions to other health promoting properties such as anti-allergic, anti-inflammatory, antimicrobial and anticancer properties (Aiyelaagbe and Osamudiamen, 2009). The ++ symbols in ethanolic extract indicates the presence of secondary alkaloids. Alkaloids have been reported to possess analgesic, antispasmodic and bactericidal, antimalarial and analgesic activities, (Okwu and Okwu, 2004; Oomah, 2003). Saponins prevent the excessive intestinal absorption of cholesterol and thus reduce the risk of cardiovascular diseases such as hypertension (Akinpelu and Onakoya, 2006) and steroids showed the analgesic properties and central nervous system activities (Abdul, et al., 2013).

Table 1. The Biochemical Compounds of *Derris elliptica* Benth.

Phytochemicals Tested	**Ethanolic Extract**
Tannins	**+**
Flavonoids	**+**
Alkaloids	**++**
Saponins	**+**
Steroids	**+**

Present (+); Absent (-)

Table 2 depicts the average zone of inhibition of bacteria tested on the three replicates with 30 ml of *Derris elliptica* ethanolic extract. It is reflected on the table that the *Derris elliptica* was more active against *Staphylococcus aureus* having the total average zone of inhibition of 24.66mm than *Escherichia coli* having the total average zone of inhibition of 20mm. “This is because cell wall of gram-negative bacteria is more complex than gram positive bacteria which make it more susceptible and impermeable” (Kumar, et al., 2006; Zaidan, et al., 2005).“Therefore, this explains why the gram-negative bacteria were more resistant to antimicrobial compounds with its effective diffusion barrier. It is not surprising to note that *E. coli* in the study was the most resistant microorganism between the bacterial strains, despite the

fact that *E. coli* developed multi drug resistance toward different kinds of antimicrobial agents" (Sader, et al., 2002). "On the other hand,
S. aureus was more susceptible bacteria of the two bacterial strains tested. Several reports suggest that *S. aureus* is the most common pathogen to cause skin infections" (Jones, et al., 2003). The zone of inhibition of the extract from Tubli applied to the bacterial species were also higher compared to the Erythromycin, an antibacterial drug which was used in several studies to fight these microorganisms (Maobe, et al., 2013).Thus, *Derris elliptica* Benth root extract has the potential to be used in pharmaceutical to combat against *S. aureus* and *E. coli.*

Table 2. Zone of Inhibition (mm) of the Growth of *Staphylococcus aureus* and *Escherichia coli* under *Derris elliptica* Root Extract.

Bacterial Species	Replication			Total	Mean
	R1	R2	R3		
Staphylococcus aureus	25	25	24	74	24.66
Escherichia coli	20	20	20	60	20

Figure 2 Shows the Zone of Inhibition of the Growth of *Staphylococcus aureus*and *Escherichia coli* under *Derris elliptica* Root Extract.

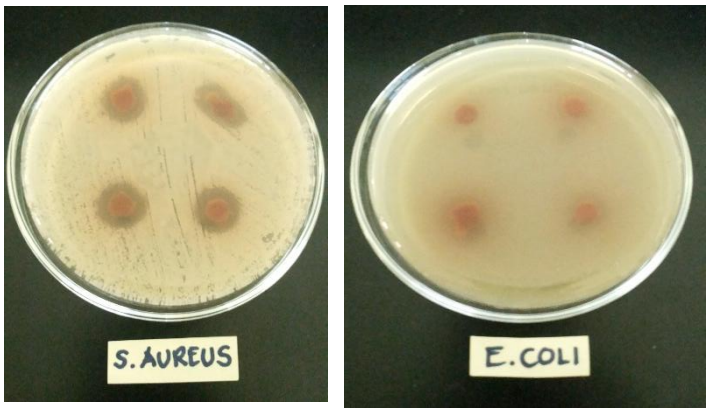

Figure 2: Zone of Inhibition of the Growth of *Staphylococcus aureus*and *Escherichia coli* under *Derris elliptica* Root Extract.

Table 3. Test of Significant Difference of the Average between *Escherichia coli* and *Staphylococcus aureus.*

Source	Type III Sum of Squares	df	MS	F	P-Value	Interpretation
Model	3025.333[a]	2	1512.667	9076.000	.000	Significant
Treatment	3025.333	2	1512.667	9076.000	.000	Significant
Error	.667	4	.167			
Total	3026.000	6				

Table 3 depicts the test of significance difference in the mean average of zone of inhibition between *E. coli and S. aureus*. The results revealed that the p-value is 0.000 which is lesser than the significant level which is 0.05. This implies that there is a significant difference between the mean average of zone of inhibition between *E. coli and S.aureus.*

5. SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

This section presents a summary of findings, conclusions, and recommendations generated from the results of the study.

5.1 Summary

The main purpose of the study was to analyze the bioactive compounds present in Tubli (*Derris elliptica* Benth) root extract, determine the result of the mean average of zone of inhibition between*Escherichia coli* and *Staphylococcus aureus* and differentiate the mean average of zone of inhibition between *Escherichia coli* and *Staphylococcus aureus*. The study used experimental method of research.

Phytochemical analysis of Tubli (*D. elliptica* Benth) revealed the presence of tannins, flavonoids, alkaloids, saponins, and steroids which contributed to the antimicrobial ability of the root extract.Results of the mean average of zone inhibition showed that Tubli(*D. elliptica* Benth) root extract has 24.66mm of the growth of*Staphylococcus aureus* and 20 mm of the growth of *Escherichia coli*. The tests revealed that Tubli (*D. elliptica* Benth) was found to be very active against the bacterial species because it has a big zone of inhibition which shows its potential to kill or inhibit the of bacteria.

Based on the findings, there is a significant difference between the mean average of zone of inhibition between *Escherichia coli* and *Staphylococcus aureus*.

5.2 Conclusions

Based on the results of the study, the following were drawn:

1.The bioactive compounds present in Tubli (*D. elliptica*) which contributed to its antimicrobial property are tannins, flavonoids, alkaloids, saponins, and steroids against Escherichia coli and *Staphylococcus aureus.*
2.The mean average of zone of inhibition of *D. elliptica* Benth root extract on *Staphylococcus aureus* is higher than the mean average of zone of inhibition of *Escherichia coli*.
3.There is significantdifference in the mean average of zone of inhibition between *Escherichia coli* and *Staphylococcus aureus*.

5.3Recommendations

Based on the outcome of the study, the following are recommended for further studies:

1.Develop research regarding antimicrobial property of other parts of the plant.
2.Thus, further research must be done to determine the efficacy of these extracts against various other pathogenic bacterial and fungal species.
3.Higher the research of this species that can make an antibiotic drug.
4.Research on other uses of *Derris elliptica* Benth.
5.Use different amounts of concentration in applying the extracts of tubli to the bacterial species.

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REFERENCES

[1] Abdul W, Mehreen G, Syed BJ, Muhammad N, Ajimal K, Rukhsana G and Asnad (2013): Phytochemical analysis of medical plants occurring in local area of Mardan. Biochemical & Analytical biochemistry. 2(4).

[2] Adekunle, A.S. & Adekunle, O.C(2009). Preliminary assessment of antimicrobial properües of aqueous extract of plants against infectious diseases. Bi010U and Medicine. 1(3):20-24.

[3] Akinmoladum AC, Ibukun EO, Afor E, Obuotor EM, Farombi EO (2007). Phytochemical constituent and antioxidant activity of extract from the leaves of Ocimumgratissimum. Sci. Res. Essay. 2: 163-166.

[4] Akinpelu DA, Onakoya TM (2006). Antimicrobial activities of medicinal plants used in folklore remedies in south-western. Afri. J.Biotechnol. 5: 1078-1081.

[5] Alam MJ and Zurek L.(2006). Seasonal prevalence of Escherichia coli 0157:H7 in beef cattle feces. J Food Prot.69(12):3018-20.

[6] Ashok, P and Upadhyaya, K. (2012). Tannins are Astringent. Journal of Pharmacognosy and Phytochemistry. ISSN 2278-4136, ZDB-Number:2668735-5, IC Journal No.:8192, Volume 1 Issue 3.

[7] Ateba CN, Bezuidenhout CC. (2008). Characterization of Escherichia coli 0157 strains from humans, cattle and pigs in the Northwest Province, South Africa. Int J Food Microbiol.128(2):181-8.

[8] Atttawadee SY, Chitchamai O, Arunporn I, Ruedeekorn W (2006): Extraction of rotenone from Derris elliptica and Derris maZaccensis by pressurized liquid extraction compared with maceration. Journal of Chromatography A. 1125 (Suppl 2):172-176.

[9] Bano, S. (2007). Chemistry of Natural Products. Department of Chemistry, Faculty of Science, JamiaHamdard, New Delhi-110062

[10] Diekema DJ, Haller MA, Schmitz FJ, Smayevsky J, Bell J, Jones RN, Beach M.(2001). Survey of infections due to Staphylococcus species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, the Western Pacific reøon for the SENTRY Antimicrobial Surveillance Progam 1997— 1999. *ClininfectDis 32* (Suppl 2): S114-S132.

[11] Doughari, J.H.; Human, I.S, Bennade, S. &NdaKdemi, P.A. (2009). Phytochemicals as chemotherapeutic agents and antioxidants: Possible solution to the control of antibiotic resistant verocytotoxin producing bacteria. Journal of Medicinal Plants Research. 3(11): 839-848.

[12] Doughari, J.H. &Obidah, J.S. (2008). Antibacterial potentials of stem bark extracts of Leptadenialancifoli.against some pathogenic bacteria. Pharmac010Uonline 3: 172-180.

[13] Gupta, R.C.(2007) Rotenone. In Veterinary Basic and Clinical Principles; Gupta, R.C., Ed; Academic: New York; pp.499-501.

[14] Habila;J.Bello;I.Dzikwe; A.Ladan;Z.andM.Sabiu (2011).BasicApplied.Science.Aust.J.,5,537.

[15] Isman MB (2006): Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annual Review Entom010U. 51:45-66.

[16] Isman MB (2006): Botanical inseticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annual Review Entom010U. 51:45-66.

[17] Krishnaiah D, Sarbatly R, Bono A (2007) Phytochemical antioxidants for health and medicine: A move towards nature. BiotechnolM01Bi01 Rev 1: 97-104.

[18] Kumar, K. and Pandey, K. (2013). Chemistry and Activities of Flavonoids: An Overview. The Science World Journal Volume 2013 (2013), article ID 162750, 16 pages.

[19] Kumar, V.P., Chauhan, N.S., Padh, H. , Rajani, M. , 2006. Search for antibacterial and antifungal agents from selected Indian medicinal plants. J. Ethnopharmacol. 107, 182—188.

[20] Ladhani, G. (2005) Staphylococcal infections in children: rational drugtherapy recommendations. from http://www.ncbi.nih. gov/pubmed/ 15871629.

[21] Lindsay JA, Holden MT (2004) *Staphylococcus aureus*: superbug super genome? Trends Microbiol 12: 378-385.

[22] Luthria, D. L. ,&Natarajan, S. S. (2009). Influence of sample preparation on the assay of isoflavones-Planta Medical, 75, 704—710.

[23] Mahajan, M. Kumar, V and Yadav S.V. (2014). alkaloids: properties, application and pharmacological effects pp. 1-36.

[24] Maobe M, Gitu L, Gatebe E, Rotich H. Karanja P, Votha M, WambuguJ, and Muingai C (2013). Antimicrobial Activities of Eight Selected Medicinal Herbs Used for the Treatmentof Diabetes,Malaria and Pneumonia in Kisii Region, Southwest Kenya. DOI: 10.5829/idosi.gjp.2013.7.1.65133

[25] Okwu DE, Josiah C (2006). Evaluation of the chemical composition of two Nigerian medicinal plants. Afri. J.Biotechnol. 5 (4):357361. [26] NAAEE. (1996). Environmental Education Materials: Guidelines for Excellence. Washington, DC: Author. IED 403 1451

[27] Oomah, D.B., 2003. Isolation, characterization and assessment of secondary metabolites from plants for use in human health. PBI Bull., 13-20.

[28] Orak HH. (2011). Antibacterial and antifungal activity of Pomegranate (*Punicagranatum L*. CV.)Pee1.AJEAFCh 10: 1958-1969. [29] Pardo, C. G. (2012). Environmental awareness, practices, and attitudes of selected UNP students. UNP Research Journal, 21, 145-164

[30] Ott, K.(2000). Rotenone. A Brief Review of its Chemistry Fate, and the Toxicity of Rotenone Formulations.

[32] Poojary;M. Vishnumurthy; K. and A. Adhikari.(2015). Extraction, Characterization and Biological Studies of phytochemicals from Mammeasuriga. Department of Chemistry, National Institute of Technology Karnataka, Surathkal 575025,India: Elsevier.

[33] Rios, J.L. &Recio, M.C. (2005). Medicinal plants and antimicrobial activity. Journal of Ethnorpharmacoloor. 00: 80-84.

[34] Sader, H.S., Jones, R.N., Silva, J.B., 2002. Slön and soft tissue infections in Latin American Medical Centers. Four-year assessment of the pathogen frequency and antimicrobial susceptibility patterns. Diagl. Microb. Infect. Dis. 44, 281— 288.

[35] Sashikala GD, Kottai AM, Satheesh DK, Rekha S, Indhumathy, (2009). Studies on the antibacterial and antifungal acüvities of the ethanol-ic extracts of Luffacylindrica(Linn) fruit. Int J Drug Dev. Res 1: 105-109.

[36] Schito GC (2006) The importance of the development of antibiotic resistance in *Staphylococcus aureus*. Clin Microbiol Infect 12 (Suppl 1): 3-8.

[37] Stauth D (2007). Studies force new view on biolog.r of flavonoids. Oregon State University.USA.
stephens JM (2003) Gourd Luffa-Luffa cylindrical, Luffaaegyptica and Luffaacutangula. J HorticSciUniv Florida 3: 19-21.

[38] Tamura, Y. Miyakoshi, M. and Yamamoto, M. (2012). Application of Saponin-Containing Plants and Cosmetics. Chapter 5.

[39] Temple, N. J. (2000). Antioxidant and disease: More questions than Trease and Evans Pharmacology. (2002). 15th edition. W.B. Saunders. London.

[40] Teng; M. (2008). Philippine Medicinal Plants. Philippines: Prelude MedicinalPlantDatabase /Accessed:http://www.stuartxchange.com/T ubli.html

[41] Visetson,S; &Milne,M. (2001). Effects of Root Extract from Derris (Derris ellipticaBenth) on Mortality and DetoxificationEnzyme Levels in the Diamondback Moth Larvae (Plutellaxylostella Linn.)Kasetsart J. (Nat. Sci.) 35 : 157 — 163.

[42] Wadood A, Ghufran M, Jamal SB, Naeem M, }Q1an A, et al. (2013) Phytochemical Analysis of Medicinal Plants Occurring in Local Area of Mardan. Biochem Anal Biochem 2: 144. doi: 10.4172/2161-1009.1000144.

[43] WHO (World Health Organization).(2005).Preventing chronic diseases a vitalinvestment. Http://www.who.int/chp/chronic_disease_report /full_report.pdf.

[44] Yao, L. H., Jiang, Y. M., Shi, J., Tomas-Barberan, F. A., Data, N., Singanusong, R., et a-1.(2004). Flavonoids in food and their health benefits. Plant Foods for Human Nutrition, 59, 113— 122.

[45] Yogisha S, (2009). In-vitro antibacterial effect of selected medicinal plant extracts. J Nat Prod 2:64-69.

[46]. Zaoutis, E.(2006)Community-acquired Methicillin resistant *Staphylococcus aureus* Infection in the Pediatric Population. http://www.medscape.com/viewaticle/578510.