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ANTIMICROBIAL ACTIVITY OF ANDROGRAPHIS PANICULATA LEAF EXTRACT

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Abstract

The increasing proliferation of diseases, with the associated drug resistance, has underscored the need for new drug development. Andrographis paniculata is one of the medicinal plants with rich active diterpenoids for the potential discovery of new drugs. It is commonly used for its anti-inflammatory, antineoplastic, antioxidant, antipyretic, anti-hypertensive, antidiabetic, and immune-boosting properties. However, virtually no research has been done to unravel the antimicrobial properties of the plant. Thus, this research was done to investigate the antimicrobial activity of Andrographis paniculata. This study made use of the experimental research design in actual laboratory setup. Disk diffusion method was used to screen the antimicrobial activity of andrographis paniculata leaf extract against Staphylococcus aureus and Escherichia coli, with amoxicillin as the control. Fresh leaves of Andrographis paniculata

were shade-dried, powered, and extracted using ethyl alcohol. The results revealed remarkable growth inhibitions of Andrographis paniculata against Staphylococcus aureus and Escherichia coli. My study demonstrates that Andrographis paniculata has antimicrobial activity against the test microorganisms. Therefore, the leaf extract can be used as an antibacterial agent for the diseases caused by these pathogens.

Keywords: *Antimicrobial activity, Andrographis paniculata (Klorostrep), Leaf extract, Staphylococcos aureus, Escherichia coli*

INTRODUCTION

One of the major challenges facing mankind from time immemorial is disease endemic. Today, several thousands of diseases have been diagnosed, with many of them still being incurable. There are many things that can cause diseases, but microorganisms are the main cause of human, animal and plant diseases. For example, it was discovered in 19th century that microbes can cause numerous infectious diseases, like flu, measles, chickenpox, tuberculosis, whooping cough, cholera, etc. Researchers also discovered in 20th century that microbes contribute also to many non-infectious chronic diseases and conditions, such as some forms of cancer, coronary artery diseases, diabetes, multiple sclerosis, and chronic lung diseases. Microorganisms are of diverse kinds—pathogenic and non-pathogenic microorganisms. As non-pathogenic microbes cause no harm in their hosts, pathogenic microbes cause lots of harms (diseases and death) in their hosts. Study shows that both infectious and non-infectious human diseases are caused by pathogenic microorganisms. In this study, therefore, two microbial species: *Staphylococcus aureus and Escherichia coli (E. coli)* have been selected to test the antimicrobial property of *Andrographis paniculata.*

Staphylococcus aureus is a facultative anaerobic Gram-positive spherical bacterium that is usually found in the human respiratory tract, gastrointestinal tract, the nasal passages (the nose), mouth,

and on the skin, as well as in the air, water, milk and sewage. Although it is not always pathogenic, it is a common cause of many illnesses, like skin infections, endocarditis, pneumonia, osteomyelitis, toxic shock syndrome (TSS), meningitis, sepsis, bacteremia, and food poisoning. On the other hand, *Escherichia coli* is a Gram-negative, facultatively anaerobic, rod-shaped bacterium, which is commonly found in the digestive tracts of humans and other warm-blooded organism (endotherms). There are many types of E. coli, and most of them are harmless. But some strains, like O157:H7, are harmful, and can cause diseases like bloody diarrhea, severe anemia, kidney failure, and urinary tract infections.

Medical science has made a remarkable advancement through the discovery of numerous drugs and medicines—antibiotics, hormones and vaccines—for the treatment of several diseases caused by pathogenic microbes, even though these pharmaceutically formulated medicines are not totally free from side effects. Nevertheless, more studies are still needed to be done to discover cure for many diseases that still remain incurable. This therefore underscores the need to involve herbal medicine in the fight against diseases, because it is cost effective with little or no side effects. To this end, different plants and herbs should be studied to harness their medicinal values. Studies have shown that plants are the major raw materials used in medical researches for the production of drugs and medicines. In fact, the use of plants in the management and treatment of diseases started with life (Agbafor, et al., 2011).[1] Right from his creation, man has depended on plants for survival. The plant kingdom called Plantae, with its various species—about 300,000 species—is the major source of food and medicine for the mankind. Man depends directly or indirectly on plants for food. Through photosynthesis, plants (green plants) convert solar energy into chemical energy (food) for the survival of man. However, man also depends on plants for medicine; both for orthodox and herbal medicine. Through phytochemical screening of plants, it has been revealed that various plants have medicinal values and properties for the treatment of different human diseases.

The Philippines is one of the countries in the world that has rich flora, with various medicinal plants. Thus, herbal medicine is recognized in the country, to the extent that lots of medicinal plants have been endorsed to be used as herbal medicine in Philippines due to their health benefits. The government through the Department of Health (DOH) launched the Traditional Medicine Program in 1992 for effective and safe use of traditional medicine in the country. This led to the signing of traditional medicine into law Republic Act 8423 (R.A. 8423), otherwise known as the Traditional and Alternative Medicine Act (TAMA) of 1997. One of the common medicinal plants found in the country is *Andrographis paniculata*. Locally known as Klorostrep in the Philippines, *A. paniculata* is also called Mahatita in India, Chuan Xin Lian in China, andrographics or King of Bitters in English. It is widely cultivated in Southern and Southeastern Asian countries, but it originated from India and Sri Lanka.

Andrographis paniculata is an erect annual herb that belongs to *Acanthaceae* family. It is extremely bitter in taste; thus, it is referred to as “King of Bitters”. It can be found in a variety of habitats, such as hillsides, plains, coastlines, and cultivated areas like wastelands, farms, and roadsides. It grows erect to a height of 30-110 cm in moist, shady places, and thrives best in a sunny location. The slender stem is dark green, squared in cross-section with longitudinal furrows and wings along the angles. The lance-shaped leaves have hairless blades measuring up to 8 cm long by 2.5 wide. The small flowers are borne in spreading racemes. The fruit is a capsule

around 2 cm long and a few cm wide. It contains many yellow-brown seeds. *Andrographis paniculata* has several traditional uses since ancient times, particularly its leaves and roots. It is frequently used in traditional medicines to treat numerous diseases including common cold, although pregnant women should not use because it can terminate pregnancy (Mayo Clinic Book of Alternative Medicine, 2010).[3] It is distributed in tropical Asian countries, often in isolated patches. It is native to South Asian country, particularly India and Sri Lanka, but has been introduced and cultivated as a medicinal plant in many parts of Asia, like the Philippines, China, Thailand, Malaysia, Hong Kong, Indonesia, etc. It is cultivated during May and June, and does best in sunny locations. It is present in 26 different polyherbal formulations in the Ayurvedic traditional health system.

As the world population grossly increases, human diseases also increase. This therefore stresses the need to research more into alternative medicine (herbal medicine), so as to augment orthodox medicine (conventional medicine). Although all parts of the plant have traditionally been used, the leaves are the most common medicinal part of the plant (Akbar, 2011).[2] The phytochemical analysis of *Andrographis paniculata*, using its leaves, reveals that the plant contains a number of bioactive constituents that make it highly medicinal. The most active constituent of the plant extract is andrographolide, which exhibits anti-inflammatory activity by inhibiting nitric oxide production and cyclooxygenase-2 expression. In mouse hepatocytes, it induced mRNA expression of P450 subfamily members, CYP1A1 and CYP1A2, in a concentration manner (Sloan-Kettering Cancer Center, 2013).[4] Other constituents discovered in the plant include: 14-Deoxy-11-dehydroandrographolide, 14-Deoxy-11-oxoandrographolide, 14-Deoxy-11, 12-didehydroandrographolide, 14-Deoxy-12-hydroxyandrographolide, 3,4-Dicaffeoylquinic acid, 5-Hydroxy-7,8,2`,3`-Tetramethoxyflavone, Diterpene lactones, Glycosides, Flavanoids, B-sitosterol, Stigmasterol, Ergosterol peroxide, Andrographine, Andrographolide, Neoandrographol ide, Panicoline, Paniculide-A, Paniculide-B, Paniculide-C. These diterpenoids possess a wide spectrum of important biological activities such as antiparasitary, cardiovascular, anti-inflammatory, phytotoxic, anticancer, and others (Veneziani, R.C.S., et al., 2017).[21]

According to Sloan-Kettering Cancer Center (2013), Andrographis has been mostly studied for the treatment of colds, flu, and upper respiratory infections.[4] It is frequently used to prevent and treat common cold and flu (influenza). Some people claim that it stopped the 1999 flu epidemic in India. Furthermore, it is used for the treatment of digestive complaints including diarrhea, constipation, intestinal gas, colic, and stomach pain. It is also used for liver conditions including an enlarged liver, jaundice, and liver damage due to medications. It can equally be used for infections like leprosy, pneumonia, tuberculosis, gonorrhea, syphilis, malaria, cholera, leptospirosis, rabies, sinusitis, and HIV/AIDS; and for skin conditions including wounds, ulcers and itchiness. However, some people use andrographis for sore throat, coughs, swollen tonsils, bronchitis, and allergies. It is also used for atherosclerosis (hardening of the arteries), and for the prevention of heart diseases and diabetes. Other uses of *Andrographis paniculata* include treatment of snake bites and insect bites, loss of appetite, kidney problems (Pyelonephritis), hemorrhoids, and an inherited condition called Familial Mediterranean Fever. It is also used as an astringent, bacteria-killing agent, painkiller, fever reducer, and treatment for worms. It might stimulate the immune system, and also improve the blood cell counts in people with HIV. Likewise, it is used for dyspepsia, flatulence, parasitic infestation of the gastrointestinal tract, diarrhea, poor digestion, hepatitis, liver insufficiency, and liver toxicity. It equally actives

fibrinolysis, and has blood pressure-lowering effect. As released by Sloan-Kettering Cancer Center (2013), andrographis exhibits antibacterial, anti-cancer, anti-inflammatory, anti-diabetic, anthelmintic, antioxidant, and immunostimulating properties.[4] However, it warned that patients should use caution before using it as it might interact with many drugs. The center insisted that andrographis should be contraindicated to patients who are taking chemotherapy drugs, cytochrome p450 substrates, antihypertensive drugs, anticoagulants. According to Sheeja (2006), extracts of *A. paniculata* exhibited potent anti-inflammatory and antioxidant effects in mice.[15] Govindarajan (2011) published that andrographis extracts have the potential to be used as a mosquito repellant.[6] Andrographis extracts and andrographolide derivatives have demonstrated modest activity in vitro against HIV (Reddy, 2005).[12]

In other words, animal and in vitro experiments using human cancer breast cell lines to investigate the potential anticancer effects of *A. paniculata* have found andrographolide responsible for the observed effects (Sukardiman, et al., 2007).[17] According to Zhang (2009), andrographis extracts have shown hypoglycemic action and beta cell protective effects for diabetes treatment in rats with streptozotocin-and alloxan-induced diabetes.[22] In his separate report, Hidalgo (2005) stated that andrographolide had demonstrated anti-inflammatory effects in several cellular systems, including prevention of phorbol ester-induced reactive oxygen species and N-formyl-methionyl-lemcyl-phenylalamine-induced adhesion in rat neutrophils, inhibition of tumor necrosis factor-induced upregulation of intercellular adhesion molecule expression, and monocyte adhesion, and activation of protein kinase pathways.[8] In antimalarial screening, Siti (2002) noted that andrographis extracts proved antimalarial effects.[16] A study revealed that the extract of *A. paniculata* blocked E. coli enterotoxin-induced secretion in rabbit and guinea pig models of diarrhea, and andrographolide and three other related diaterpenes were responsible for the action.

On the other hand, some studies have found that prolonged high-dosage of *A. paniculata* can cause toxicity in the liver and testicles (decreased sperm counts and motility). According to Mayo Clinic Book of Alternative Medicine (2010), andrographis is an abortifacient, and should not be used by pregnant women, because it can terminate pregnancy.[3] Few other adverse reactions reported with the use of *A. paniculata* include headache, dizziness, flank pain, decreased urine output, rash, diarrhea, fatigue, pruritus, altered taste, decreased sex drive, nausea, vomiting, diarrhea, and others. (Poolsup, 2004 and Sloan-Kettering Cancer Center, 2013).[4,10]

However, it is regrettable that despite over 300,000 species of plant kingdom with their respective medicinal phytochemicals necessary for the treatment of human diseases, many diseases still remain incurable. This is as a result of man's failure to harness the medicinal values of those plants. Hence, plant kingdom has become a target for multinational drug companies and research institutes, both in the Philippines and other countries of the world, for the discovery of new drug precursors. One of such plants with rich medicinal potentials is *Andrographis paniculata*. But unfortunately, only little is known about its antmicrobial activity. Thus, this research is conducted to investigate the antimicrobial property of *Andrographis paniculata* leaf extract against *Staphylococcus aureus* and *Escherichia coli*.

STATEMENT OF THE PROBLEM

This study generally aims to determine antimicrobial activity of *Andrographis paniculata* leaf

extract against *Staphylococcus aureus* and *Escherichia coli.*

Specifically, this study seeks to answer the following questions:
What is the antimicrobial property of *Andrographis paniculata* leaf extract in terms of the zones of growth inhibition of the following test microorganisms: *Staphylococcus aureus* and *Escherichia coli?*

SCOPE AND DELIMITATION

This study was delimited to the determination of antimicrobial activity of *Andrographis paniculata* leaf extract using ethyl alcohol as solvent in terms of the diameters of growth inhibition of the two test microorganisms, namely: *Staphylococcus aureus and Escherichia coli.* Only the fresh leaves of *Andrographis paniculata,* which were collected from Nagsupotan, San Juan, Ilocos Sur, Philippines and shade-dried, were used in this experiment. The research was conducted at the Natural Science Research Unit, School of Natural Sciences, Saint Louis University, Baguio City, Philippines, from June, 2014 to February, 2015.

CONCEPTUAL FRAMEWORK

The experimental paradigm which was used in the study is presented below:

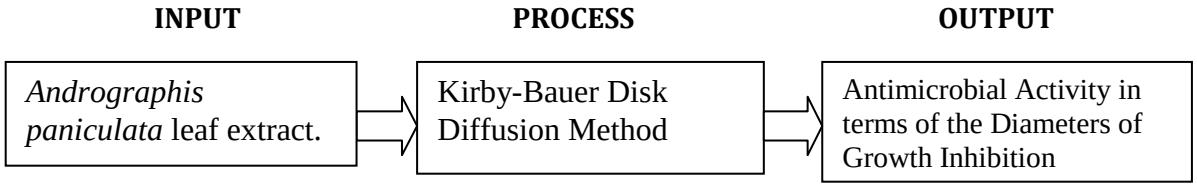

Figure 1: The Experimental Paradigm

As diagrammatically represented in the paradigm above, the antimicrobial property of the leaf extract of *Andrographis paniculata* using ethyl alcohol was tested on the two test microorganisms, namely: *Staphylococcus aureus, Escherichia coli*. The diameters of growth inhibition of the above mentioned organisms were used to determine the antimicrobial property of the plant.

MATERIALS AND METHODS

Collection of Plant Materials

Fresh and healthy leaves of *Andrographis paniculata* were collected from Nagsupotan, San Juan, Ilocos Sur, Philippines.

Preparation of Plant Extract

The collected leaves were washed with water, rinsed with distilled water, dried in a shade, chopped, and finally ground into fine powder using a mechanical grinder. The extraction of the *Andrographis paniculata* leaves was done using classic techniques for solvent extractions of plant materials. The powder of *Andrographis paniculata* leaves (100g) was soaked in 300ml of 95% ethyl alcohol in an Erlenmeyer flask for 48 hours, and then was filtered using a filter paper in a Buchner funnel, with the leaf residues discarded. The filtrate was concentrated to 20 ml, and the concentration of the plant extract was computed, expressed as grams plant material per ml of the extract. The extract yields were weighted, properly labeled, and stored in a tightly stoppered container in the refrigerator at 0-5^{o}C.

Screening for Antimicrobial Activity
The Kirby-Bauer Disk Diffusion Method was used for the antimicrobial activity screening.

Preparation of Nutrient Broth and Agar Media
The weighed 5.0g peptone, 3.0g yeast extract, and 2.5g sodium chloride were dissolved in 100 ml of distilled water. To prepare the nutrient agar medium, 16.0g of agar was added to the solution of nutrient broth. The media were both sterilized at 15 psi at 121°C for 20 minutes.

Preparation of Sabouraud Glucose Broth and Agar Media
The weighed 10.0g neopeptone and 40.0g glucose were dissolved in 1000 ml of distilled water. Then, to prepare sabouraud glucose agar, 5.0g of sabouraud dextrose agar (SDA) was dissolved in 1000 ml of distilled water, and was heated to dissolve it completely.

Preparation of 0.5 McFarland Standards
0.5 ml 0.048 M $BaCl_2$ (1.175 % w/v $BaCl_2$ $2H_2O$) was mixed with 99.5 ml of H_2SO_4 (1% v/v), and 5 ml of the solution was poured into a screw-capped tube of equal size to the tubes used to prepare the culture suspension. The tube was then sealed and stored in the dark at room temperature.

Preparation of Sterile Isotonic Saline-Tween 80 Solution
To prepare the isotonic saline solution, the weighed 0.85 g of sodium chloride was dissolved in 100 ml of distilled water, and then 0.1 ml of Tween 80 solution was added to the isotonic saline solution, and was thoroughly mixed to dissolve the Tween 80. Afterwards, the Tween solution was sterilized at 121°C for 15 minutes, and then was cooled before use.

Preparation of Inocula
Bacteria
A loopful of pure bacterial culture was inoculated into a 5 ml nutrient broth and the suspension was incubated for 18-24 hours at 35-37° C till the turbidity matches that of McFarland 0.5.

Filamentous Fungi
A loopful of pure fungal culture was inoculated in 50 ml of Sabouraud Glucose Agar Plate. It was incubated for 18 hours for 2-3 days at 30°C, and was shaken vigorously afterwards for one minute. Five loopfuls of the inoculums were immersed in 5 ml of sterile isotonic saline-Tween 80 solution contained in a screw-capped tube. The test tube was shaken vigorously again for one minute, and the turbidity was compared with that of 0.5 ml McFarland standard. Sterile isotonic saline was used as diluents for yeast cells. This yeast inoculum was used to swab the agar plates for the screening. The above suspensions were used within 15 minutes after the turbidity has been adjusted.

Adjusting the Turbidity of the Inocula
The turbidity was adjusted by adding sterile saline solution or culture broth and subsequently comparing the resulting turbidity to the standard in case the bacterial suspension did not appear to be of the same turbidity as the McFarland.

Preparation of Agar Plates
Approximately 15ml of melted Nutrient agar or Sabouraud Glucose Agar was poured into dry

and sterilized Petri dishes. The media were allowed to solidify before use.

Seeding of Agar Plates

A sterile cotton swab was dipped into the bacterial broth suspension or saline-Tween 80 spore suspension, with excess inoculum removed by rotating the swab several times against the wall of the test tube above the fluid level. The entire surfaces of the agar were streaked evenly in all directions. The swabbed plates were allowed to stand for 5 minutes.

Placement of Disks

Using sterilized forceps, sterile 6 mm disk was dipped into the plant extract or control, and was laid and pressed gently (to ensure maximum full contact of the dish with the agar medium) on the estimated center of the quadrant of the Petri dish. Three quadrants of the Petri dish were for the plant extracts and the fourth was for the control.

Incubation and Observation of the Plate

Plates were inverted and incubated for 30 minutes of the inoculation at 35-37^{o} C for bacteria for approximately 18 hours and at 27^{o} C for the fungus for two days.

Reading and Interpretation

The plates were inverted when taking the reading. The diameter of each zone of inhibition was measured to the nearest tenth millimeter with a vernier caliper. For the purposes of standardization, the following interpretative range of standard zones was adopted:

Diameter of Inhibition (mm)	Inhibitory Activity
19 mm and above	+++ very active
14-19 mm	++ active
10-13 mm	+ partially active
Below 10	-inactive

RESULTS

The result analyses in terms of diameters of inhibition of the test organisms and the antimicrobial activity of *Andrographis paniculata* leaf extract are respectively shown in tables 1 and 2 below.

Table 1: Zone of inhibition of *Staphylococcus aureus* as affected by *Andrographis paniculata* extract using Disc Diffusion method

T	Sample Treatment	Replication (measured in mm)*			Mean (mm)
		I	II	III	
T-1	Klorostrep extract (10 µL)	0	0	0	**0**
T-2	Klorostrep extract (20 µL)	0	0	0	**0**
T-3	Klorostrep extract (40 µL)	11	11	11	**11**
T-4	Klorostrep extract (80 µL)	14	14	14	**14**
T-5	Amoxicillin,25 µg (control)	37	37	37	**37**
			Grand Mean		**20.67**

*18-24 hours incubation period at 35^{0}C±2^{0}C in Mueller-Agar

Interpreting the zone of inhibition:

<10 mm: Inactive

10-13
mm: Partially active
14-19 mm: Active
>19 mm: Very active

From table one, it can be seen that the zones of inhibition of *Andrographis paniculata* leaf extract against *Staphylococcus aureus* in the three replications of T_1 and T_2 (10 µL and 20 µL) were all zero mm (0 mm), indicating no inhibition on the growth of the bacterium. However, in T_3 and T_4 (40 µL and 80 µL), the zones of inhibition of the organism increased to 11mm and 14 mm respectively in the three replications. This therefore shows that the diameters of inhibition of *S. aureus* increased with the increase in the quantity of *Andrographis paniculata* extract. On the other hand, as an antibiotic for bacterial infections, amoxicillin as a control sample in T_5 had the highest zone of inhibition of 37 mm against *S. aureus*.

Hence, the antimicrobial property of the leaf extract was inactive on *Staphylococcos aureus* in T_1 and T_2, with mean of zero (0) mm respectively. In T_3, it was partially active with mean of 11 mm, and active in T_4 with mean of 14 mm, while the antimicrobial property of amoxicillin in T_5 was very active on the test organism with zone of inhibition of 37 mm.

Table 2: Zone of inhibition of *Escherichia coli* as affected by *Andrographis paniculata* extract using Disc Diffusion method

T	Sample Treatment	Replication (measured in mm)*			Mean (mm)
		I	II	III	
T-1	Klorostrep extract (10 µL)	0	0	0	**0**
T-2	Klorostrep extract (20 µL)	0	0	0	**0**
T-3	Klorostrep extract (40 µL)	8	8	8	**8**
T-4	Klorostrep extract (80 µL)	11	11	11	**11**
T-5	Amoxicillin,25 µg(control)	18	18	18	**18**
			Grand mean		**12.33**

*18-24 hours incubation period at $35^0C \pm 2^0C$ in Mueller-Agar

Interpreting the zone of inhibition:

<10 mm: Inactive
10-13 mm: Partially active
14-19 mm: Active
>19 mm: Very active

From table two, it can be seen that the zones of inhibition of *Andrographis paniculuta* leaf extract against *E. coli* in the three replications of T_1 and T_2 (10 µL and 20 µL) were all zero (0) mm in the three replications, indicating no inhibition on the growth of the bacterium. But in T_3 and T_4 (40 µL and 80 µL), the zones of inhibition of the organism increased to 8 mm and 11 mm respectively in the three replications. This therefore shows that the zones of inhibitions of *E. coli* increased with the increase in the quantity of *Andrographis paniculata* extract. However, amoxillin as the control sample in T_5 had the highest zone of inhibition of 18 mm against *E. coli*.

Hence, the antimicrobial property of the leaf extract was inactive on *Escherichia coli in* T_1 and T_2 with mean of zero (0) mm respectively. In T_3, it was still inactive with mean of 8 mm, and partially active in T_4 with mean of 11 mm, while in T_5, it was active on the test organism with mean of zone of inhibition of 18 mm.

DISCUSSION

In this research, ethyl alcohol extract of *Andrographis paniculata* was tested for zone of inhibition and Minimum Inhibitory Concentration against a Gram-positive bacterium, *Staphylococcos aureus* and a Gram-negative bacterium, *Escherichia coli.*

The results indicate remarkable inhibitory actions against *Staphylococcos aureus and Escherichia coli* (Tables 1 and 2), which means that *Andrographis paniculata* leaf extract posseses antimicrobial activity against *Staphylococcos aureus and Escherichia coli.*

With this, my study has therefore proved that *Andrographis paniculata* leaf extract can be used as an antibacterial agent to treat any diseases caused by these two pathogens.

This discovery of my research is a ray of hope, given the incessant proliferation of diseases and continually increased drug-resistant strains of pathogens. Medicinal plants have been the source of treatment for human infections. (Khan,2013)[12] This is because such plants are rich in phytochemicals like terpenoids, flavonoids, alkaloids, tannins, and others, which are responsible for their medicinal properties. According to previous studies, the phytochemical analysis of *Andrographis paniculata*, using its leaves, revealed that the plant contains a number of bioactive constituents that make it highly medicinal. The most active constituent of the plant extract is andrographolide. Other constituents discovered in the plant include: 14-Deoxy-11-dehydroandrographolide, 14-Deoxy-11-oxoandrographolide, 14-Deoxy-11, 12-didehydroandrographolide, 14-Deoxy-12-hydroxyandrographolide, 3,4-Dicaffeoylquinic acid, 5-Hydroxy-7,8,2`,3`-Tetramethoxyflavone, Diterpene lactones, Glycosides, Flavanoids, B-sitosterol, Stigmasterol, Ergosterol peroxide, Andrographine, Andrographolide, Neoandrographolide, Panicoline, Paniculide-A, Paniculide-B, Paniculide-C. (Hossain, S.M, et al., 2014)[10]. It is believed that these secondary metabolites are responsible for the pharmacological potentials of *Andrographis paniculata*.

From the results, the ethyl alcohol extracts showed more significant antibacterial activity when in a larger quantities than in lesser quantities, as shown in Tables 1 and 2. Thus, this showed that the diameter of growth inhibition increased with an increasing quantity of the leaf extract. From the increasing trend of the outcomes, it can be inferred that the inhibition zones of the test microorganisms would have increased to a more active level if higher quantities (100 µL and above) of the leaf extract were used. Moreover, the results indicated that *Andrographis paniculata* had more inhibitory effects on *Staphylococcus aureus* than on *Escherichia coli.* The results contradict the claims of Gurupriya, S., et al., (2016) that the *Andrographis paniculata* possessed higher antibacterial activity against *E. coli* than *S. aureus.*[8] This contradiction may be due to the fact that my study used ethyl alcohol leaf extracts of the plant with the maximum extract quantity of 80 uL, unlike theirs that made use of chloroform stem extracts with maximum quantity of 150 uL. It can also be because of errors in study designs, which includes inappropriate analytical methods.

This study believes that the diterpenoids of *Andrographis paniculata* are responsible for its antimicrobial potentials. This is in line with the conclusion of Mishra, P.K., et al., (2013), even though they believed that the terpenoids present in methanol extract of *A. paniculata* leaves was responsible for the antibacterial actions of the plant in their research.[13] Although this research made no use of methanol leaf extracts but ethyl alcohol extracts only, the results still recorded antibacterial actions against the test organisms. Therefore, it is believed that the diterpenoids

present in the ethyl alcohol leaf extracts have the same potentials as those present in methanol extracts, and so, are responsible for the antibacterial activity recorded in the results of this study.

In as much as this research was successful with significant results, it still had some limitations. Pharmacological testing of *Andrographis paniculata* was beyond the scope of this study. Again, the fact that this study made use of only the leaves of the plant, ethyl alcohol as the extracting solvent, 80 uL as the maximum quantity of the leaf extract, and only two microorganisms (*S. aureus* and *E. coli)*, could have limited the generalizability of the results of the study. To this end, this study recommends that subsequent studies should make use of other parts of the plant, other extracting solvents, larger quantity of the extract (100 uL and above), and other microorganisms.

CONCLUSION

This study concludes that *Andrographis paniculata* leaf extract has antibacterial activity against *Staphylococcos aureus* and *Escherichia coli.* Therefore, it can be used as an antibiotic agent for infections caused by the test pathogens.

CONFLICTS OF INTEREST

The author has none to declare

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REFERENCES

1. Agbafor, K.N., et al, (2011). Phytochemical Analysis and Antioxidant Property of Leaf Extracts of Vitex doniana and Mucuna pruriens. Retrieved from https;//doi.org/10.1155/2011/459839
2. Akbar, S. (2011). "*Andrographis paniculata:* a Review of Pharmacological Activities and Clinical Effects". Alternative Medicine Review.
3. Andrographis paniculuata, Mayo Clinic Book Of Alternative Medicine, 2010, p.47.
4. Andrographis, Sloan-Kettering Cancer Center, 2013
5. Coon, J.T. (2004). "*Andrographis paniculata* in the Treatment of Upper Respiratory Tract Infections: a Systematic Review of Safety and Efficacy. Plant Med.
6. Govindarajan, M., et al. (2011). Mosquito Adulticidal and Repellent Activities of Botanical Extracts Against Malarial Vector, Anopheles Stephensi Liston (Diptera: Culicidae), Asian Pac J Trop Med, Retrieved from doi:10.1016/S1995-7645(11)60233-X
7. Guevara, B. Q. (2005). A Guidebook to Plant Screening: Phytochemical and Biological,UST Publishing House; pp.23-60.
8. Gurupriya, S., et al., (2016). Antibacterial activity of *Andrographis paniculata* stem extract.International Journal of Science and Engineering Research, Vol 7, Issue 8, ISSN2229-5518
9. Hidalgo, M.A., et al., (2005). Andrographolide interferes with binding of nuclear factor-Kb to DNA in HL-60-derived neutrophilic cells. Br. J. Pharmaco., 144:680-685.

10. Hossain, M.S., et al., (2014). *Andrographis paniculata* (Burm. f.) Wall ex Nees: A Review of Ethnobotany, Phytochemistry, and Pharmacology. The Scientific World Journal. Retrieved from http://doi.org/10.1155/2014/274905.
11. Khan, S. U., et al., (2013). Antimicrobial potentials of medicinal plants extract and their drivered silver nanoparticles: A focus on honey bee pathogen. Saudi Journal of Biological Sciences, Vol 26, Issue 7, Pg 1815-1834. Retrieved from http//doi.org/10.1016/j.sjbs.2018.02.2010
12. Lalitha, M.K. (2005). "Manual on Antimicrobial Susceptibility Testing". Indian Assoc. of Med. Microbiol.
13. Mishra, P.K., et al., (2013). Antibacterial activity of *Andrographis paniculata* (Burm. f.) Wall ex Nees leaves against clinical pathogens. Journal of Pharmacy Research, Vol. 7, Issue 5, Pg 459-462. Retrieved from http://doi.org/10.1016/j.jopr.2013.05.009
14. Poolsup, N.,et al., (2004). Andrographis paniculata in the symptomatic treatment of uncomplicated upper respiratory tract infection:systematic review of randomized controlled trials, J Clin Pharm Ther. Retrieved from doi:10.1046/j.1365-2710.2003.00534.x
15. Quinto, E.A. et. Al. (2004). "Microbiology. In B.Q. Guevara (Ed.), *A Guidebook to Plant Screening: Phytochemical and Biological"* (pp. 65-102). Espana, Manila: Research Center for the Natural Sciences, University of Santo Tomas
16. Reddy, V.L.N., et al., (2005). A New Bis-Andrographolide Ether from Andrographis paniculata nees and Evaluation of Anti-HIV Activity, Nat Prod Res. 2005 (3):223-30. Retrieved from doi:10.1080/14786410410001709197
17. Righi A. A., et. al., (2013). "Comparative Chemistry of Propolis from Eight Brazilian Localities. *Evidence-Based Complementary and Alternative Medicine"* 2013, Article ID 267878
18. Saxena, R. C., et. Al. (2010). A Randomized Double Blind Placebo Controlled Clinic Evaluation of Extract of *Andrographis paniculata* (Kalmcold) in Patients with Uncomplicated Upper Respiratory Tract Infection. Phytomedicine
19. Sheeja, K, Shihab, P.K, and Kuttan, G (2006). Antioxidant and Anti-Inflammatory Activities of Plant Andrographis Paniculata Nees, Journal of Immunopharmacology and Immunotoxicology, Vol. 28, 2006 Issue 1, pages 129140. Retrieved from http://doi.org/10.1080/08923970600626007
20. Siti Najila, M.J., et al., (2002). The Screening of extracts from Goniothalamus scortechinii,
21. Aralidium pinnatifidum and Andrographis paniculata for anti-malarial activity using the lactate dehydrogenease assay. J Ethnopharmacol Retrieved from http://doi:org/10.1016/S0378-8741(02)00183-6.
22. Sukardiman, H., et al., (2007). Apoptosis Inducing Effectof Andrographolide on TD-47 Human Breast Cancer Cell Line,Afri.J.Traditional, Complementary and Alternative Medicines. Retrieved from www.africanethnomedicines.net.
23. Thomson, M. (2012). www.sund-forskning.dk/artikler/andrographis-paniculata-o
24. Todar, K. (2006) "The Bacterial Flora of Humans". Todar's Online Textbook of Bacteriology. University of Wisconsin. 2006. Retrieved from http://textbookofbacteriology.net/normalflora.html
25. Varma, A., et. al. (2011). "Andrographolide: a New Plant-derived Antineoplastic entity on horizon. Evid Based Complement Alternat Med.
26. Veneziani, R.C.S., et al., (2017). Antibacterial Potential of Diterpenoids, Studies in Natural

Products Chemistry. 2017

27. Zhang, Z., et al. (2009). Hypoglycemic Action and Beta Cell Protective Effects Andrographolide Analogue for Diabetes Treatment, Journal of Translational Medicine 7(1:62). Retrieved from doi:10.1186/1479-5876-7-62