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Antimicrobial Potentials of *Dolichos lablab* Linn. (Hyacinth Bean)

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ABSTRACT

Dolichos lablab Linn. is used as a traditional medicine against different ailments. To elucidate its antimicrobial activity, the plant underwent phytochemical screening which indicated that the leaf and pod extracts contain triterpenes, alkaloids, flavonoids, sterols, tannins, and glycosides, which provide a broad antimicrobial spectrum against different microorganisms.

Plant extracts were analyzed to test the antibacterial activities against gram-negative *(Escherichia coli)* and gram-positive *(Staphylococcus aureus)* bacteria. Minimum Inhibitory Concentrations (MIC) were measured. *Dolichos lablab* leaf and pod extracts showed the same inhibition zone (10.00 mm) against *Escherichia coli* and *Staphylococcus aureus*. The leaf and pod extracts exhibited complete inhibitory activity at 25% concentrations as compared to the effects of *Amikacin* for *Escherichia coli* and partial and complete inhibitory activities of the leaf and pod extracts, respectively, for *Staphylococcus aureus* as compared to *Oxacillin*. The inhibitor activity of *Dolichos lablab* is found to be positive, indicating that *Dolichos lablab* leaf and pod extracts partially inhibited *Aspergillus niger*. The ethanolic extracts of the leaf and pods showed minimal activity against *Aspergillus niger*. *Dolichos lablab* may not possess antifungal activities against *Candida albicans* since it did not produce any inhibition zones.

The leaf and pod extracts showed MIC (Minimum Inhibitory Concentration) values of (.39 mg/ml) for *Escherichia coli* and (1.56 mg/ml) for *Staphylococcus aureus*. Results showed that the ethanolic extracts of the leaf and pod exerted a greater antibacterial effect on *Escherichia coli* than *Staphylococcus aureus*. Low MIC indicates that it is an excellent candidate as antimicrobial agents against *Escherichia coli* and *Staphylococcus aureus*.

Keywords: antimicrobial activity, inhibitory activity, minimum inhibitory concentration, phytochemical.

INTRODUCTION

Worldwide, infectious diseases emanating from microorganisms such as bacteria, fungi, viruses, and parasites are hazardous to public health due to the growing resistance of many microorganisms to currently available antibiotics. The increasing use of herbal medicines has proven to be effective in the treatment of certain diseases.

The development of "green medicine" sweeping across the world, the use of traditional medicine and medicinal plants in most developing countries, including the Philippines, has been widely observed as a normative basis for the maintenance of good health.

Because of these characteristics of plants, a law in the Philippines was promulgated to promote the use of herbal medicine as alternative drug. Republic Act 8423 known as the Traditional Alternative Medicinal Act (TAMA) has established the Philippine Institute of Traditional Alternative Health Care (PITAHC) to promote the use of traditional medicinal practices. This law, being referred to as Alternative Medicine Law is expected to promote and to develop the country's traditional or folk knowledge of healing and medicinal practices, especially in the use of medicinal plants (PITAHC, 2018).

In the Philippines, the Philippine Institute of Traditional and Alternative Health Care (PITAHC), in partnership with the private groups and organizations have broadened the use of medicinal plants as gamut of traditional medicine, complementary and alternative approaches.

Herbal medicine has become popular in the treatments of minor ailments and on account of increasing costs of personal health care that affected the usage of herbal drugs and cures (Department of Health, 2012). Region1, being a member of PITACH, has joined efforts to conduct researches in different areas to address the global health challenges and to support the ASEAN 2020 Vision.

To the Ilocano, traditional healers and community members can easily give a diagnosis. There is a higher chance of finding successful traditional remedies from plant material used in the treatment of such infections. Plant species such as "Lagundi and "Acapulco" are efficient as these had been scientifically validated (Pagadian, 2012). Synthetic medicines are continuously emerging, readily available, and can effectively solve health problems, especially to less fortunate patients. To a great number of poor people these medicines are beyond their means because of their high cost.

Many studies on flower and leaf extracts of *Dolichos lablab* purpureus were conducted to evaluate their antimicrobial activity. During the series of tests against *Staphylococcus aureus*, it was found that these two extracts exhibited antimicrobial activity and inhibition caused by flower extracts of *Dolichos lablab* was higher than that of the leaf extracts (Dilara, 2014).

Dolichos lablab Linn. (Hyacinth Bean) is not only an attractive plant, but also has numerous claims on its medicinal values and nutrient content, especially its pods. The pods are a good source of calcium, iron, vitamin E, and other minerals. The leaves of *Dolichos lablab* in poultice form are used to treat diarrhea, and to cure wounds and boils. There is a need for such study to come up with technologies to utilize its potentials. As one of the Ilocano vegetables, the so-called "lost crop" *Dolichos lablab* given its many attributes, potential uses, and adaptation, has attracted attention in the scientific literature.

The study aims to evaluate the antimicrobial potentials of leaf and pod extracts of *Dolichos lablab* on four selected test organisms. The results from the *Dolichos lablab* extracts tested will provide a preliminary scientific justification for the traditional medicinal uses of this ethnic remedy, an essential step towards its acceptance and development as an alternative therapeutic agent.

FRAMEWORK

The widespread use of herbal remedies and preparations obtained from indigenous plants using local traditions and beliefs is still the "backbone" of health care. As defined by the World Health Organization (WHO), health is a state of complete physical, mental, and social well- being, and not merely the absence of disease or infirmity.

The Third World Nations have relied on the therapeutic properties of plants. Most of the collected plant materials which are freshly used include the leaves, flowers, stems, berries, and roots for their therapeutic or medicinal use. Due to the high cost involved in manufacturing modern medicines, many people simply do not have the financial resources to pay for them. As a result, they are forced to use natural herbs (WHO, 2010).

Plants are primary sources of new natural medicinal products. Extracted from plants and used globally, 74 percent were discovered. Studies showed that the chemical substances amongst medicinal plants are responsible for combating human diseases (WHO, 2010).

The use of plants as herbal remedies may provide mankind a very safe, effective, and quality traditional medicine (Atienza, 2019). It will also facilitate the discovery of new sources of drugs and promote sustainable use of medicinal plant resources.

The practice of using herbs in medicine dates back at least from India, China, Japan, and Singapore. Even in Europe and North America, the use of herbal medicine extended back more than 150 years ago and has been based on antibacterial activity. Also the basis for the development of new drugs came from the processing of the plant extracts. Plants produce a diverse array of secondary compounds that may be effective in combating bacterial pathogens. Plants, therefore, are good sources of investigation for potential antimicrobial pathogens commonly known to cause diseases.

The compound present in plants typically resulting from the combination of secondary products is an effective way to improve the efficacy of such herbal medicine. Compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol viewed as potential sources of new drugs, antibiotics, insecticides and herbicides (Crozier, 2017). These are synthesized and deposited in specific percentage or in all parts of the plant (Joseph & Raj, 2017). Generally, the leaves are the favorable storage site for desired compounds. With these, plants have provided a source of inspiration for novel drug compounds as plant derived medicines for human health and well-being (Masoko & Eloff, 2017).

Today, scientists incorporate past knowledge and continually advance scientific research and testing of plants for their efficacy as source of alternative herbal medicine. Ground breaking documentation is being proven for herbal medicines treating diseases with little to no treatment options. It is a known fact that commercially produced modern medicines are very expensive. Marginalized people from developing countries could not afford them. Because of this situation, many of them are forced to look for natural herbs that are affordable and readily available alternatives (Herbtreatment.com, 2017). Industrialized countries like China, India, and other western countries practice herbal medicine systems. Among the systems are Chinese herbology, Ayurveda medicine, Roman and Greek herbs, and Shamantic herbs. These show that there is now a second look at herbal medicines to address affordability and availability of medicines. Moreover, the remoteness and lack of reliable modern facilities in the rural communities also enhances the dependence of plants for medicine.

The antimicrobial activity of the extracts can be attributed to the high triterpenes and sterols and other phytochemicals found in the *Dolichos lablab* Linn. pod extracts (Shrestha et al., 2015).

As in the case of *Dolichos lablab* there have been a number of reviews in recent years typically scattered over a range of journals, reports, or manuscripts. Many of which will probably be never published; therefore, will remain inaccessible to most researchers. As one of the traditional Ilocano vegetables, the so called "lost crop" *Dolichos lablab* given its many attributes, potential uses, and adaptation has attracted certain attention in science literature.

Previous studies have identified and documented numerous medicinal plants for treatment of many diseases that can be determined by means of phytochemical screening. (Mojab & Crozier, as cited in Joseph & Raj (2017), Crozier, 2017), further mentioned the presence of numerous chemicals including naturally occurring alkaloids and other nitrogen containing compounds. Most of the plant materials collected include the use of leaves, flowers, stems, berries, and roots for their therapeutic or medicinal use. In addition, many of these have been found to exhibit in vitro antimicrobial properties which are largely viewed as potential sources of new drugs, antibiotics, insecticides and herbicides.

Secondary metabolites are organic compounds that are directly involved in the normal growth, development, or reproduction of organisms. These secondary metabolites play a significant role in plant defense against herbivory and other interspecies defenses. For humans, these secondary metabolites are used as medicines, flavorings, and recreational drugs (Bhatt & Weis, 2017). The effects of these plant materials result from the combination of the secondary metabolites in plants such as alkaloids, steroids, tannins, and phenol compounds which are viewed as potential sources of new drugs, antibiotics, insecticides and herbicides.

An antimicrobial is an agent that kills microorganisms or inhibits their growth. Antimicrobial medicine can be grouped according to the microorganisms where they act primarily like antibacterial used against bacteria and antifungal used against fungi. They can also be classified according to their functions. Agents that kill microbes are called microbial while those that merely inhibit their growth are called micro biostatic (Arranz, 2015).

Antimicrobial resistance is an urgent threat to public health and modern medical therapy. Globally, an estimated 700,000 people die each year from antimicrobial- resistant infections At Penn Medicine , leaders initiated a project to leverage health IT to improve antibiotic prescribing with the aim of ensuring the right patient gets the right antibiotic at the right time (University of Pennsylvania Medical Center Guidelines for Antibiotic Use).

In screening new antimicrobials or antibiotics, evaluation of biological activity is an important assessment of susceptibility of pathogens to the microbial agent. Antimicrobial susceptibility is used in pathology to determine the resistance of certain microbial strains to different antimicrobials and in pharmacology research to determine the efficacy of novel antimicrobials from biological extracts against microorganisms (Das, 2014).

This study is also related to the investigation of Dahake et al. (2017). His study tried to determine the antimicrobial potential of the plant by using different extracts of *Dolichos lablab* against pathogenic bacteria. They have similarities since the present study will use the same strains of bacteria used by Dahake et al. However, the study differs from the cited study because other parts of the will be used.

Numerous studies have identified compounds within herbal plants that are effective antibiotics (Basil, as cited in Parekh & Chanda, 2007). The use of plant extracts can be of great significance in therapeutic treatments. Many Asian countries most especially in the Philippines, India, Africa and the Caribbean consume this plant *Dolichos lablab* as green vegetables and use it to cure different ailments in humans and in animals (Snafi, 2017).

OBJECTIVES OF THE STUDY

This study aimed to evaluate the antimicrobial activity of *Dolichos lablab* leaf and pod extracts. Specifically, the study sought to (1) determine the qualitative phytochemicals present in the leaf and pod extracts of *Dolichos lablab* (2) determine the antimicrobial activity of the leaf and pod extracts of four test organisms namely: *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus niger* and *Candida albicans* in terms of total mean zone of inhibition, reactivity, and inhibitory activity; and (3) assess the Minimum Inhibitory Concentration (MIC) *Dolichos lablab* leaf and pod extracts.

METHODS

In this study, the plant samples were collected and properly selected, not damaged by diseases, insects, weather, or mechanical injury.

Herbarium specimens of *Dolichos lablab* were prepared and sent to the University of the Philippines Baguio Herbarium for proper authentication by a taxonomist.

Time and Place of the Study

All procedures were conducted in an accredited level II laboratory in DOST – ITDI Taguig, Metro Manila. The extraction process, confirmatory test, and microbiological screening passed through the biosafety committee at the DOST – ITDI, Bicutan Taguig Rizal.

Preparation of the Plant Extract

The procedure for the stock plant was adapted from the method described by Guevara (2005). Seven hundred grams of the ground fresh leaves and pods of *Dolichos lablab* were weighed and treated with sufficient ninety percent ethyl alcohol. The flask was stoppered and was soaked for 48 hours, filtered, and concentrated in a rotary evaporator under atmospheric pressure and a temperature below 50°C until there was no more alcohol present.

Bacterial Strains

Standard bacterial strains of Gram-positive and Gram-negative species were used; *Staphylococcus aureus* (ATCC-25923), *Escherichia coli* (ATCC- 25922), and fungal strains: *Aspergillus niger* (ATCC 16404) and *Candida albicans* (ATCC 90028).

Antimicrobial Property Testing

The Kirby- Bauer disk diffusion test was conducted on the two bacteria; *Staphylococcus aureus* (ATCC-25923), *Escherichia coli* (ATCC- 25922), and on the two fungal strains: *Aspergillus niger* (ATCC 16404) and *Candida albicans* (ATCC 90028). *Amikacin* 30µg was used as a positive control for *Escherichia coli*, and *Oxacillin* 10µg for *Staphylococcus aureus*.

The antimicrobial susceptibility test of the selected pathogens was done by Disc diffusion method using the Kirby- Bauer disk diffusion technique. was performed on the two bacteria by inoculating them onto Mueller- Hinton agar. containing the test compound at different concentrations (25 %, 50 %, 75 %, 100 %) were placed on the agar surface using sterile swab sticks. The sterile disc of diameter 6mm were impregnated with extract solutions (0.1mg/ml and

0.2mg/ml) and placed onto the cultured Muller Hinton agar plates. Inculcated plates were incubated at 37 0 C for 24 hrs. The plates were read by taking the measurement zone of inhibition around each cell. The diameter of zone inhibition of the bacteria was recorded in milliliters. The assay was done in triplicates and checked with the control plate.

Minimum Inhibitory Concentration

Delost (2004) suggested using the dilution method for essential plant extracts which exhibit capable antibacterial activity. Different concentrations of *Dolichos lablab* leaf and pod extract are prepared by the serial dilution method (two-fold dilution) subsequently assayed against the test organism with series tubes containing 1ml of MH (Mueller Hinton) broth to achieve the final dilutions of 100, 50, 25, 12.5, 6.25, 3.25, 1.56, 0.78, and 0.39. 0.19 (mg/ml) until the final concentration was approximately 1x105 to 1x106 cfu/ml of the chosen isolated *Staphylococcus aureus* and *Escherichia coli*. The media were incubated for approximately 48 hrs. After the incubation period, the tubes were analyzed for the presence or absence of growth. The microbial growth inhibition zone was measured after incubation at 37 degrees. The lowest concentration or dilution of the plant extract in serial dilution sequence that results in the absence of observable growth is reported as minimal inhibitory concentration, MIC. To analyze the data, graph pod prism was used.

RESULTS AND DISCUSSION

Phytochemical Analysis of *Dolichos lablab* Linn. of Leaf and Pod Extracts

Qualitative tests were done as a preliminary phytochemical screening of the leaf and pod extracts of *Dolichos lablab* for the presence of secondary metabolites. Table 1 shows the results of the phytochemical screening analysis of *Dolichos lablab* leaf and pod extracts.

The phytochemical study revealed the presence of various phytochemicals in the leaf and pod extracts. The leaf and pod extracts are rich in triterpenes and sterols, alkaloids, flavonoids, saponins, glycosides, and tannins.

Table 1

Qualitative Phytochemical Analysis of Dolichos lablab Linn Leaf and Pod Extracts

Phytochemicals	Leaf extracts	Pod extracts
Sterols	++	+++
Triterpenes	+	+++
Flavonoids	+	+
Alkaloids	+	+
Saponins	+	++
Glycosides	+	+
Tannins	+	+

Legend: (+) = traces
(++) = moderate
(+++) = abundance

The leaves of the plants contain moderate amounts of sterols, showed traces of flavonoids, alkaloids, and saponins. *Dolichos lablab* leaf extracts displayed the presence of a moderately high amount of sterols; and trace amounts of triterpenes, flavonoids, alkaloids saponins, glycosides, and tannins. On the other hand, pod extracts contain abundant levels of sterols and triterpenes; moderate amounts of saponins; and trace amounts of flavonoids, alkaloids, glycosides, and tannins (Table 1).

The active chemical constituents of the medicinal plants have been found to have biological activities. The flavonoids have useful properties, including anti-inflammatory, antibacterial property against *Staphylococcus aureus*, and *B. subtilis* (Sattapongan, 2011). Other compounds, including triterpenes, sterols, alkaloids, were present in most selected remedies.

Ramakrishna (2015) as cited in Satiskumaran (2016), Parvin (2016) showed that Dolichos lablab also contains bioactive constituents such as alkaloids, tannins, saponins. It reduces sugar through its leaf extracts, which are responsible for significant antibacterial activity of the crude extracts of this plant. Tannins are polyphenolic compounds that have a bitter and astringent taste. They are known for their laxative effect and are also thought to be responsible for antidiarrheal and antibacterial activities. Their capacity has been developed as a way to defend against external attacks from predators. These phytochemical compounds identified in the leaf and pod may be responsible for the biological activities shown by *Dolichos lablab* which are known for their use as traditional medicine.

Table 2

Total Mean Zone of Inhibition and Reactivity of Escherichia coli Subjected to Different Concentrations of Dolichos Lablab Linn Leaf and Pod Extracts

Concentration Control / *Dolichos lablab* Extract (%)	Leaf Extract		Pod Extract	
	Zone of Inhibition (mm)	Reactivity	Zone of Inhibition (mm)	Reactivity
Sample-free disc (negative control)	0	0	0	0
(100)	10.00	2	10.00	2
Sample-free disc (negative control)	0	0	0	0
(75)	10.00	2	10.00	2
Sample-free disc (negative control)	0	0	0	0
(50)	10.00	2	10.00	2
Sample-free disc (negative control)	0	0	0	0
(25)	10.00	2	10.00	2
Amikacin 30ug (positive control)	14.02	3	14.02	3

Test Reference: United States < Biological Reactivity Test In vitro Pharmacopoeia

Legend:

> 19 – very active	*4– severe (Zone extends to greater than 10 mm beyond specimen)*
14–19 – active	*3– moderate (Zone extends from 5 mm to 10 mm beyond specimen)*
10–13 – partially active	*2– mild (Zone is limited under the specimen)*
< 10 – Inactive	1– slight (some have malformed or degenerated cells under the specimen)
	0– none (no detectable zone around specimen is observed)

The total mean zone of inhibition of the leaf and pod extract treatment against *Escherichia coli* in all concentrations is 10.00 mm. *Amikacin* 30ug, served as a positive control for the leaf (*Escherichia coli*) with a total mean zone of inhibition of 14.02 mm. Sample free disc served as negative control registered a zero total mean of inhibition. This shows that the leaf and pod extracts are partially active in controlling the growth of *Escherichia coli*.

The reactivity of *Escherichia coli* to *Dolichos lablab* in 100, 75, 50, and 25 percent concentration was mild, which means that there was a limited zone of inhibition under the specimen.

Amikacin 30µg, which served as a positive control for *Escherichia coli*, produced a moderate reactivity (3) against the test organisms. Noticeably, *Dolichos lablab* leaf and pod extracts produced a mild (2) reactivity against *Escherichia coli*, which means that *Escherichia coli* is moderately sensitive to *Dolichos lablab* leaf and pod extracts.

Table 3 presents the effect of *Dolichos lablab* extracts on the growth of *Escherichia coli*, as shown by the inhibitory activity of different concentrations.

Table 3

Inhibitory Activity of Different Concentrations of Dolichos lablab Linn Leaf and Pod Extracts Against Escherichia coli

Dolichos lablab Linn Extract Concentrations (%)	Inhibitory Activity	
	Leaf extracts	Pod extracts
25	+++	+++
50	+++	+++
75	+++	+++
100	+++	+++

Legend: +++ – complete
++ – partial
+ – slight

In terms of leaf extract treatment, there was (+++), complete inhibitory activity of *Dolichos lablab* to *Escherichia coli* at 25, 50, 75, 100 percent concentrations. The results, therefore, suggest that the leaf extracts prevented the growth of *Escherichia coli* at a minimum inhibitory concentration of 25 percent. In terms of pod extract treatment, there was also (+++), complete inhibitory activity of *Dolichos lablab* to *Escherichia coli* at 25, 50, 75, 100 percent concentrations. The positive inhibitory of *Dolichos lablab* leaf and pod extracts completely prevented the growth of the organism, *Escherichia coli*.

The complete inhibition of *Dolichos lablab* maybe due to the presence of a moderate number of compounds with antimicrobial potentials like triterpenes, sterols, alkaloids, flavonoids, saponins, glycosides, and tannins. The effectivity of the leaf and pod extracts of *Dolichos lablab* in suppressing microbial growth with various potency as a result of the thin cell wall of *Escherichia coli* leading to the rapture of the bacterial cell wall. The presence of the phytochemicals present in the extracts supports the ability of the plant to inhibit the growth of bacteria and fungi and its ability to scavenge free radicals. Parekh and Chanda (2007) reported that flavonoids were among the phytochemicals present in plants that exhibited moderate to strong antimicrobials.

The inhibitory activity of *Dolichos lablab* refers to the action of its leaf and pod extracts to interfere with the growth of the test organisms.

Escherichia coli is also considered as an extra pathogen in the intestines. Its high incidence can be associated with morbidity, mortality, and increasing antimicrobial resistance (Russo & Johnson, 2015). As reported, *Escherichia coli* caused the loss of billions of health care and thousands of lives in the USA.

United States < Biological Reactivity Test In vitro Pharmacopoeia (2007) test reference book which describes the drugs, chemicals and medicinal preparations which is used as standard for drug strength, quality, purity and safety of essential medicines.

Table 4

Total Mean Zone of Inhibition and Reactivity of Staphylococcus aureus Subjected to Different Concentrations of Dolichos Lablab Linn. extracts

Concentration Control / *Dolichos lablab* Linn Extract (%)	Leaf Extract		Pod Extract	
	Zone of Inhibition (mm)	Reactivity	Zone of Inhibition (mm)	Reactivity
Sample-free disc (negative control)	0	0	0	0
(100)	10.00	2	10.00	2
Sample-free disc (negative control)	0	0	0	0
(75)	10.00	2	10.00	2
Sample-free disc (negative control)	0	0	0	0
(50)	10.00	2	10.00	2
Sample-free disc (negative control)	0	0	0	0
(25)	10.00	2	10.00	2
Oxacillin (positive control)	23.68	4	23.68	4

Test Reference: United States < Biological Reactivity Test in Vitro Pharmacopoeia

Legend:

> 19 – very active
14–19 – active
10–13 – partially active
< 10 – Inactive

4– severe (Zone extends to greater than 10 mm beyond specimen)
3– moderate (Zone extends from 5 mm to 10 mm beyond specimen)
2– mild (Zone is limited under the specimen)
1– slight (some have malformed or degenerated cells under the specimen)
0– none (no detectable zone around specimen is observed)

The total mean zone of inhibition of the leaf and pod extract treatments against *Staphylococcus aureus* in all concentrations is 10.00 mm. Meanwhile, *Oxacillin*, served as a positive control, has a total mean zone of inhibition of 23.68 mm. The sample free disc served as a negative control, showed a zero-total mean of inhibition. This indicates that the leaf and pod extracts are partially active in controlling the growth of *Staphylococcus aureus*.

The reactivity of *Staphylococcus aureus* to *Dolichos lablab* in 100, 75, 50, and 25 percent concentration was mild (2), which means that the zone of inhibition was limited under the specimen. *Oxacillin*, which served as a positive control for *Staphylococcus aureus*, produced a severe reactivity (4) against the test organisms.

Table 5

Inhibitory Activity of Different Concentrations of Dolichos lablab Linn. Leaf and Pod Extracts Against Staphylococcus aureus

Dolichos lablab Extract Concentrations (%)	Inhibitory Activity	
	Leaf extracts	Pod extracts
25	++	+++
50	++	+++
75	++	+++
100	++	+++

Legend: +++ – complete
++ – partial
+ – slight

The minimal ability of the leaf extracts demonstrated by its partial inhibition of the growth of *Staphylococcus aureus*. Although there is a mean zone of inhibition of the leaf extracts against *Staphylococcus aureus*, there are still surviving cells in it. This can be attributed to the presence of flavonoids and other phytochemicals present in the plant since they are known to exert an antimicrobial effect. However, the amount of these compounds is not enough to warrant the antimicrobial effect against *Staphylococcus aureus*. The amount of phytochemicals present in the leaf extract is not enough to deplete the thick and compact dense walls of *Staphylococcus aureus* (Augusto, 2011).

In terms of pod extract treatment, the inhibitory activity of *Dolichos lablab* refers to the ability of the pod extracts to interfere with the growth of the test organisms. There was a complete inhibitory activity (+++) of *Dolichos lablab* pod extracts against *Staphylococcus aureus*. The pod extracts of *Dolichos lablab* prevented the growth of gram-negative bacteria at a minimum inhibitory concentration of 25 percent. The complete inhibition of the *Dolichos lablab* pod extracts can be attributed to the moderate number of antimicrobial phytochemicals.

Staphylococcus aureus is a gram-positive, non-motile, round-shaped coccus that forms into clusters. According to Dr. Franklin D. Lowly, as cited by Mandal (2016), *Staphylococcus aureus* remains a very potent and dangerous infectious bacterium.

As shown in Table 6, *Dolichos lablab* leaf and pod extracts had a total mean of 10.00mm zone of inhibition, and 18.40 against the standard drug, *Clotrimazole*. *Dolichos lablab* leaf and pod extracts possess a partially active and mild (2) reactivity against test organism, *Aspergillus niger*. The leaf and pod extracts were partially active against *Aspergillus niger* fungal strains, which suggests that the extracts partially inhibited the growth of *Aspergillus niger*.

Table 6

Total Mean Zone of Inhibition and Reactivity of Aspergillus niger Subjected to Different Concentrations of Dolichos Lablab Linn. extracts

Concentration	Leaf Extract		Pod Extract	
Control / *Dolichos lablab* Linn Extract (%)	Zone of Inhibition (mm)	Reactivity	Zone of Inhibition (mm)	Reactivity
Sample-free disc (negative control)	0	0	0	0
(100)	10.00	2	10.00	2
Sample-free disc (negative control)	0	0	0	0
(75)	10.00	2	10.00	2
Sample-free disc (negative control)	0	0	0	0
(50)	10.00	2	10.00	2
Sample-free disc (negative control)	0	0	0	0
(25)	10.00	2	10.00	2
Clotrimazole (positive control)	18.40	3	18.40	3

Test Reference: United States < Biological Reactivity Test in Vitro Pharmacopoeia

Legend:

> 19 – very active	*4– severe (Zone extends to greater than 10 mm beyond specimen)*
14–19 – active	*3– moderate (Zone extends from 5 mm to 10 mm beyond specimen)*
10–13 – partially active	*2– mild (Zone is limited under the specimen)*
< 10 – Inactive	1– slight (some have malformed or degenerated cells under the specimen)
	0– none (no detectable zone around specimen is observed)

Table 7 presents the result of the antifungal activity of *Dolichos lablab* leaf and pod extracts in terms of inhibitory activity against *Aspergillus niger*. The inhibitory activity of *Dolichos lablab* is found to be positive, indicating that *Dolichos lablab* leaf and pod extracts partially inhibited the organism, *Aspergillus niger*. The tested fungi, *Aspergillus niger*, showed minimal ability of the leaf and pod extracts of *Dolichos lablab* using ethanol extract. *Aspergillus niger* is the most common fungal species which can produce mycotoxins.

Table 7

Inhibitory Activity of Different Concentrations of Dolichos lablab Linn. Leaf and Pod Extracts Against Aspergillus niger

Dolichos lablab Linn Extract Concentrations (%)	Inhibitory Activity	
	Leaf extracts	Pod extracts
25	++	++
50	++	++
75	++	++
100	++	++

Legend: +++ – complete
++ – partial
+ – slight

Nasrin (2017) reported that the tested fungi *Aspergillus nigers* showed moderate to good antifungal activity with N-hexane extract *Dolichos lablab*. *Dolichos lablab* leaf and pod extracts indicated significant differences in their effects on *Aspergillus niger*. The present study on *Dolichos lablab* Linn may not reveal the same antifungal characteristics with the previous study conducted by Nasrin (2017). Findings are also related to the choice of extracting solvent, which may result in the different active constituents present in the extract and the different levels of essential constituents responsible for the antifungal activity of *Dolichos lablab*. The minimal ability of the leaf and pod extracts to control the growth of *Aspergillus niger* can be attributed to the fewer antibacterial phytochemicals (+) caused by the differences in the active constituents of *Dolichos lablab*.

Table 8

Total Mean Zone of Inhibition and Reactivity of Candida albicans Subjected to Different Concentrations of Dolichos Lablab Linn. Extracts

Concentration	Leaf Extract		Pod Extract	
Control / *Dolichos lablab* Linn Extract (%)	Zone of Inhibition (mm)	Reactivity	Zone of Inhibition (mm)	Reactivity
Sample-free disc (negative control)	0	0	0	0
(100)	0	0	0	0
Sample-free disc (negative control)	0	0	0	0
(75)	0	0	0	0
Sample-free disc (negative control)	0	0	0	0
(50)	0	0	0	0
Sample-free disc (negative control)	0	0	0	0
(25)	0	0	0	0
Clotrimazole (positive control)	17.00	3	17.00	3

Test Reference: United States < Biological Reactivity Test in Vitro Pharmacopoeia

Legend:

> 19 – very active
14–19 – active
10–13 – partially active
< 10 – Inactive

4– severe (Zone extends to greater than 10 mm beyond specimen)
3– moderate (Zone extends from 5 mm to 10 mm beyond specimen)
2– mild (Zone is limited under the specimen)
1– slight (some have malformed or degenerated cells under the specimen)
0– none (no detectable zone around specimen is observed)

Dolichos lablab leaf and pod extracts manifested no zone of inhibitions and produced zero reactivity against *Candida albicans*. This inhibition zone is considered inactive.

Dolichos lablab may not possess antifungal activity for controlling *Candida albicans*. This implies that *Candida albicans* is not sensitive to *Dolichos lablab* extracts which means that *Dolichos lablab* extracts are unable to control the growth of *Candida albicans*. Antimicrobial studies revealed that for an agent to be considered antibacterial, it must exhibit an inhibition zone greater than 10 mm (Saad Ibnsouda Koraichi, (2016). A larger zone of inhibition generally means that the antimicrobial is more potent, while no zone of inhibition indicates resistance.

Table 9

Inhibitory Activity of Different Concentrations of Dolichos lablab Linn. Leaf and Pod Extracts Against Candida albicans

Dolichos lablab Extract Concentrations (%)	Inhibitory Activity	
	Leaf extracts	Pod extracts
25	-	-
50	-	-
75	-	-
100	-	-

Legend: +++ – complete
++ – partial
+ – slight
– – negative

As seen from the results in Table 9, leaf and pod extracts exhibited a negative inhibitory activity against *Candida albicans*. *Dolichos lablab* may not possess antifungal activities for controlling *Candida albicans* since it did not produce any inhibition zones. The lack of inhibitory activity of *Candida albicans* to *Dolichos lablab* extracts can be attributed to the absence of phytochemicals responsible for the antifungal activity against *Candida albicans*.

Nasrin (2017) found that antimicrobial activity against fungi strains of *Candida albicans* and *Aspergillus niger* demonstrated moderate to good antifungal activity. The present study on *Dolichos lablab* may not reveal the same antifungal characteristics with the previous study of Nasrin (2015). As seen from the result, the leaf and pod extracts exhibited a zero (0) zone of inhibition against *Candida albicans*. The negative results can be accounted for the limitations of the biological activity of *Dolichos lablab* extracts against *Candida albicans*.

Compean and Ynalvez (2014) concluded that the antibacterial and antifungal activities of plants were due to the presence of glycosides, steroids, tannins, and polyphenols and the effect of the plants may be due to the disruption of proteins in the bacterial membrane.

Table 10

Minimum Inhibitory Concentration (MIC) Values of Dolichos lablab Linn. Leaf and Pod Extracts

Sample/Organism	MIC (mg/ ml)
Leaf Extract	
Escherichia coli	0.39
Staphylococcus aureus	1.56
Pod Extract	
Escherichia coli	0.39
Staphylococcus aureus	1.56

Table 10 presents the minimum inhibitory concentration (MIC) values of *Dolichos lablab* leaf and pod extracts. Results of the antimicrobial activity of *Dolichos lablab* leaf and pod extracts suggest that the plant extracts were capable of inhibiting the strain of *Escherichia coli* and *Staphylococcus aureus*. The leaf and pod extract of *Dolichos lablab* inhibited the growth of *Escherichia coli* at the lowest MIC of (0.39 mg/ ml). On the other hand, leaf and pod extracts showed a higher MIC of (1.56 mg/ml) against *Staphylococcus aureus*. *Escherichia coli* is more sensitive to the leaf and pod extracts than *Staphylococcus aureus*. The low MIC values suggest that the plant extracts at the lowest concentration can inhibit the growth of *Escherichia coli* and *Staphylococcus aureus*, which justifies their essential role in the antibacterial activity. All the leaf extracts were found active against tested Gram-negative bacteria, *Escherichia coli*. The positive control also shows antimicrobial effect. The inhibition of the bacterial strains by the leaf and pod extracts may be attributed to the presence of the secondary metabolites such as triterpenes, sterols, alkaloids, flavonoids, saponins, glycosides and tannins. Doughari (2016) reported that *Tamarindus indica* Linn. showed low MIC (0.12mg/ml.) against *Escherichia coli* and *Staphylococcus aureus*. Results of the antimicrobial activity of the extracts may explain the presence of phytochemicals present in the leaf extracts such as tannins, flavonoids, alkaloids, that serve as defense mechanisms against predation by many organisms.

On the other hand, *Dolichos lablab* partially inhibited *Staphylococcus aureus*. The minimal ability of the leaf extracts to partially inhibit the growth of *Staphylococcus* can be attributed to the presence of flavonoids and other phytochemicals.

The low MIC (< 12 mg/ml) of the leaf and pod extracts that inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* might be due to the presence of phytochemicals. The findings of this investigation strongly support the theory on the MIC that *Dolichos lablab* leaf and pod extracts to have a very high antimicrobial activity even at low concentrations.

Thus, the low MIC values for other bacteria is an indication of the efficacy of the plant *Tamarindus indica Linn.* as antimicrobial agent (Arranz, 2015). Gram-negative bacteria is one of the most susceptible to the leaf and pod extracts of *Dolichos lablab*. The extracts showed good inhibitory activities against this pathogen. Consequently, the leaf and pod extracts were able to inhibit the growth of *Escherichia coli* completely. Therefore, *Dolichos lablab* leaf and pod extracts can serve as a good candidate as antimicrobial agent against *Escherichia coli*.

The low MIC values suggest that the plant extracts at the lowest concentration can inhibit the growth of *Escherichia coli* and *Staphylococcus aureus*, which justifies their important role in the antibacterial activity. The demonstration of antimicrobial activity against both gram-positive and gram-negative bacteria indicates the presence of phytochemicals in the leaf and pod extracts of *Dolichos lablab*.

CONCLUSIONS

The present study demonstrates the antimicrobial potential of *Dolichos lablab*. The observed inhibition and low MIC of *Dolichos lablab* extracts suggest that it can suppress the growth of *Escherichia coli* and *Staphylococcus aureus*. This further confirms the inhibitory effect of *Dolichos lablab* extracts on the test organisms, which implies that it could be a potential antibacterial agent. A further quantitative phytochemical analysis is highly recommended to determine the most active compound of the plant. Pharmacokinetic and pharmacodynamics studies of the plant's leaf and pod extracts are recommended as they have promising potentials as antimicrobials.

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