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Study of Antimicrobial Activity of *Dolicus lablab* Leaf Extract

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ABSTRACT

Nature has been source of medicinal plants from thousands of years. In traditional medicine practice, the leaf of *Dolicus lablab,* commonly known as 'hyacinth bean' is said to have a significant medicinal value. In Ancient Arab medical practice the leaf of *Dolicus lablab* were said to be used in treatment of infections. Hence our present study aimed to test both anti bacterial and antifungal activities of methanol, ethanol, aqueous extracts of *Dolicus lablab* against three strains of fungi (*Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Candida albicans*.) and four strains of bacteria (*Escherichia coli, Pseudomonas aeruginosa*, *Bacillus subtilis, Staphylococcus epidermidis)* using agar well diffusion method. Among the three extracts, methanolic extract showed very potential antifungal and antibacterial activity ,with a highest zone of inhibition against fungi, *T. mentagrophytes* (30mm) and two Gram(-) bacteria, *E. coli, P. aureginosa*, with a Zone of inhibition at a range 15-19mm and 8-11mm.

Keywords: *Dolicus lablab* leaf, Antibacterial activity, Antifungal activity, Zone of inhibition.

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INTRODUCTION

According to World Health Organization (WHO), the increase of resistance to antibiotics by bacterial pathogens is a growing problem in both developed and developing countries[1]. Serious infections caused by microorganisms have become resistant to commonly used antibiotics which is the major global health care problem in 21st century. Infectious diseases are still one of the leading causes of death in the world [2,3]. Nature has been a source of medicinal agents for thousands of years and the original source of many important pharmaceuticals currently in use have been plants used by indigenous people[5]. The use of crude extracts of plant parts and phytochemicals, of known antimicrobial properties, can be of great significance in the therapeutic treatments. In recent years, a number of studies have been conducted in various countries to prove such efficiency. Many plants have been used because of their antimicrobial traits which are due to the secondary metabolites synthesized by plants[6]. *Dolicus lablab* belongs to the family Fabaceae (alt. Leguminosae). It is a large twiner. Leaves are 3-foliolate; leaflets are 5-15 cm long, ovate, acute, base cuneate or deltoid. Leaves are emmenagogue and reputed alexipharmic. *Dolicus lablab* has great potential as medicinal legume. Among the legumes, it constitutes an important source of therapeutic agents used in the modern as well as traditional systems of medicine. It has been found to possess significant medicinal properties like it can tonify the spleen and stomach, relieve internal fever, infantile malnutrition and anticancer etc,. Young and tender pods are used as vegetables in India and tropical and warm temperate Asia. It is also been known for its use as a green manure and produces edible young pods, dried seeds, leaves and flowers[11,13]. The leaves of *Dolicus lablab* have reported for its antimicrobial activity on various species of bacteria and fungi[23-25]. Fresh leaves powdered and mixed with lime are rubbed over ringworm to cure (yusuf et al., 2009). *Dolicus lablab* is also indicated as a very potent antifungal agent in the Unani medical practice, which is a very ancient Arab medical practice. In relation to the above strategy we have observed that there are very few works done on the antimicrobial activity of this plant. The present study aimed to evaluate the antimicrobial activity of different solvent extracts of *Dolicus lablab* leaf.

MATERIALS AND METHOD

Medicinal plant material collection, processing &extraction

Fresh leaves of *Dolicus lablab* were collected from various areas of Medak district, Andhra Pradesh, India and authenticated by botanist, Central Research Institute of Unani Medicine, Hyderabad. The collected leaves were washed thoroughly with tap water followed by distilled

water. The leaves were shade dried in dark at room temperature for few days and then homogenized to fine powder using a mechanical blender and stored in an airtight container. The ethanol, methanol and aqueous extracts of leaves of *Dolicus lablab* were prepared by dissolving 10gm of fine powder of medicinal plant in 50 ml of ethanol, methanol and water respectively. The contents were kept in orbitary shaker for 48 hour, and then the extract was filtered and concentrated under vacuum to obtain solid extracts. Then the extract was stored under refrigeration at 4^{o}C for further studies.

Phytochemical screening

Secondary metabolites are identified in the extracts of *Dolicus lablab* by using standard methods.1 mg of each extract was dissolved in 100 ml of the respective solvent and filtered through Whattman filter paper No.1. Thus, the filtrates obtained were used as test solutions for the screening. The results of the qualitative analysis[28-30] were given in table-1.

In-vitro antifungal assay

Three fungal strains were selected to assess susceptibility patterns against the *Dolicus lablab* leaf extracts. The fungal strains are, *Trichophyton rubrum* (Nfcci no: 2544), *Trichophyton mentagrophytes* (Nfcci no: 2545), *Candida albicans*.These strains were obtained as pure cultures from NFCCI (national fungal culture collection of India), Agharkar research institute, Pune. These cultures were inoculated on to sabouraud dextrose broth and incubated at 24^{0}- 28°(room temperature)for five days. *In vitro* antifungal activity was done using agar well diffusion method[31]. 0.1 ml of microbial culture was seeded in 25 ml molten Sabourand's Dextrose Agar butts, mixed, poured into sterile Petri plates and allowed to solidify. The wells were bored with 6 mm borer in seeded agar. 0.1ml of known concentrations (0.25, 0.50,1,2 mg/ml) of, ethanolic, methanolic and aqueous extracts of leaves of *Dolicus lablab* was added in each well. Plates were kept at 10ºC - 15 ºC as a period of pre diffusion for 30 minutes. After normalization at room temperature, the plates were incubated at room temperature for 24 – 48 hours for the growth of fungal strains. The antifungal activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm in table-2.

In-vitro antibacterial assay

Antimicrobial activity was tested against *B. subtilis, S. epidermidis, E. coli, and P. aeruginosa*. These microbial strains were isolated from clinical samples obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India, and maintained in microbiology lab at M.L.R Institute of pharmacy, Dundigal, Hyderabad, A.P., India. They were sub cultured on nutrient agar for every 15 days and maintained on nutrient agar slants at 40^{0}C. Fresh inoculums were taken for the test.

The *in vitro* antibacterial activity of the extracts was determined by agar well diffusion assay. Strains of bacteria were inoculated into 10 ml of sterile Nutrient broth in respective conical flasks, and incubated overnight at 37ºC in rotatory shaker. The cultures were swabbed on the surface of sterile Mueller Hinton agar (Hi-media) plates using a sterile cotton swab. 4 agar wells were prepared with the help of sterilized cork borer with radius 6 mm. Different concentrations of ethanolic, methanolic and aqueous extracts of leaves of *Dolicus lablab* were tested from0.25-0.90mg/ml for their activity towards bacterial strains. DMSO is used as a control and Gentamycin is used as a standard drug. Using a micropipette, 100µl of known concentration of plant extract, control and standard (supernatant) were added to the each well of the plate. The plates were incubated in an upright position at37ºCfor 24 hours. The diameter of inhibition zones measured in mm and the results were depicted in table-3.

Minimum inhibitory concentration (MIC) determination

The minimum inhibitory concentration of methanolic leave extracts was determined against *E.coli, B. subtilis, T. rubrum, T. mentagrophytes,* were found using serial dilution technique. Dilution series was set up as 20, 40, 60, 80,100µg/ml of nutrient broth media. The tubes were examined for visible turbidity after incubation. The lowest concentration which didn't show any growth of test organism after macroscopic evaluation was determined as MIC.

RESULTS AND DISCUSSIONS

Table 1: Results of Phytochemical analysis

S.No	Extract	Flavonoids	Saponins	Alkaloids	Phenols	Terpinoids
1	Aqueous	+	+	-	+	-
2	Methanolic	+	+	+	-	+
3	Ethanolic	+	+	-	+	-

Table 2: Antifungal activity of *Dolicus lablab* leaf extracts

Test sample Drug/Extract	Concentrations(mg/ml)	Diameter of Zone of inhibition (mm)		
		T.rubrum	*T.mentagrophytes*	*C.albicans*
Nistatin	0.25	10	15	21
	0.50	12	18	24
	1.0	15	19	25
	2.0	17	20	26
Aqueous	0.25	16	13	17
	0.50	18	15	19
	1.0	19	18	21
	2.0	21	24	22
Methanolic	0.25	15	14	16
	0.50	18	19	18
	1.0	21	21	19

	2.0	24	30	24
Ethanolic	0.25	10	13	13
	0.50	12	17	15
	1.0	14	18	18
	2.0	17	19	20

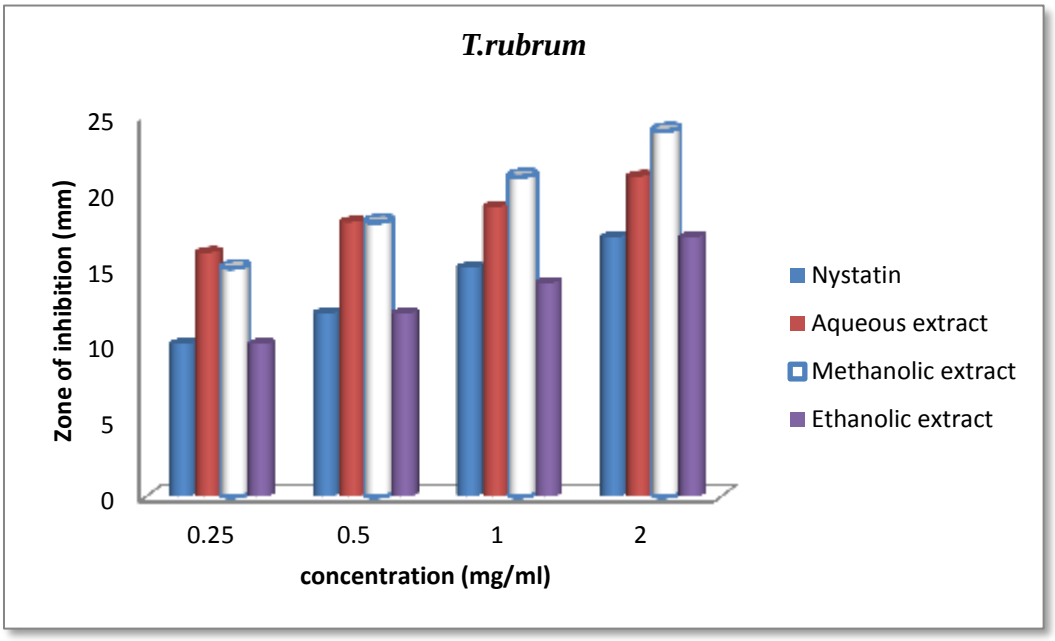

Figure1: Graphical representation of anti fungal activity of *Dolicus lablab* leaf extracts against *T.rubrum*.

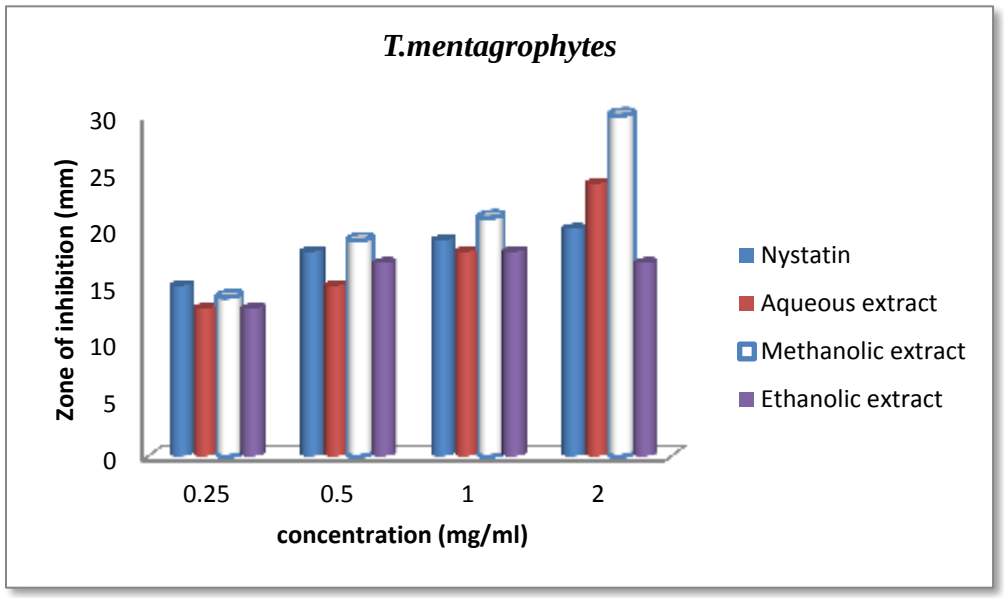

Figure-2 Graphical representation of antifungal activity of *Dolicus lablab* leaf extracts against *T.mentagrophytes*

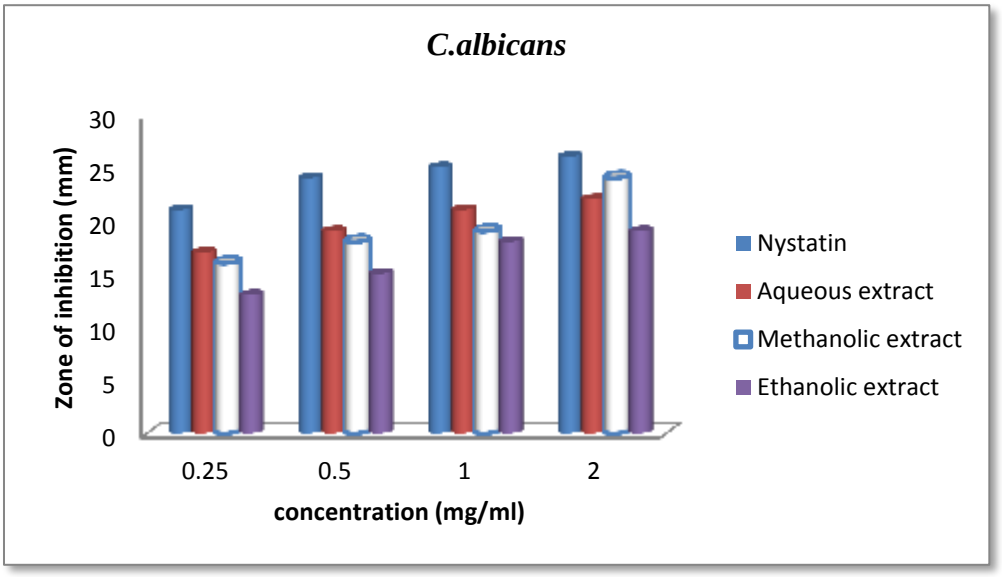

Figure-3 Graphical representation of antifungal activity of *Dolicus lablab* leaf extracts against *C. albicans.*

Table 3: Anti bacterial activity of *Dolicus lablab* leaf extract

Test sample Drug/Extract	Concentrations mg/ml	Diameter of Zone of inhibition (mm) *E.coli*	*P.aeruginosa*	*S.epidermidis*	*B.subtilis*
Gentamicin	0.05	14	10	15	14
	0.5	18	12	20	22
	0.7	18	12	21	23
	0.9	20	15	24	23
Aqueous	0.5	NA	NA	NA	8
	0.7	2	4	NA	10
	0.9	3	5	NA	11
Methanolic	0.5	15	8	NA	NA
	0.7	18	11	NA	3
	0.9	19	11	NA	4
Ethanolic	0.5	3	NA	5	13
	0.7	8	NA	9	15
	0.9	12	NA	13	18

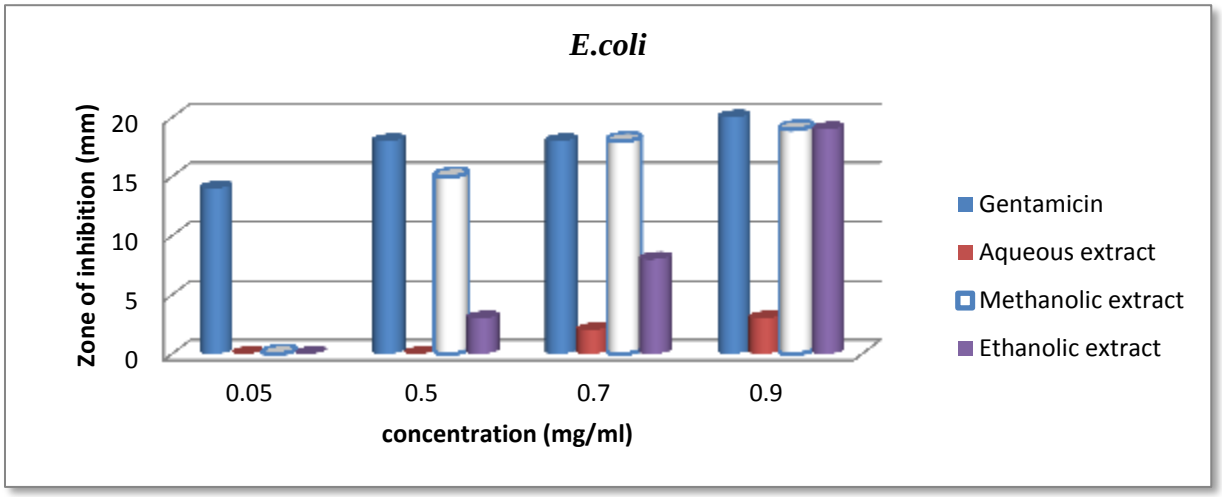

Figure-4 Graphical representation of anti bacterial activity of *Dolicus lablab* leaf extract against *E.coli*

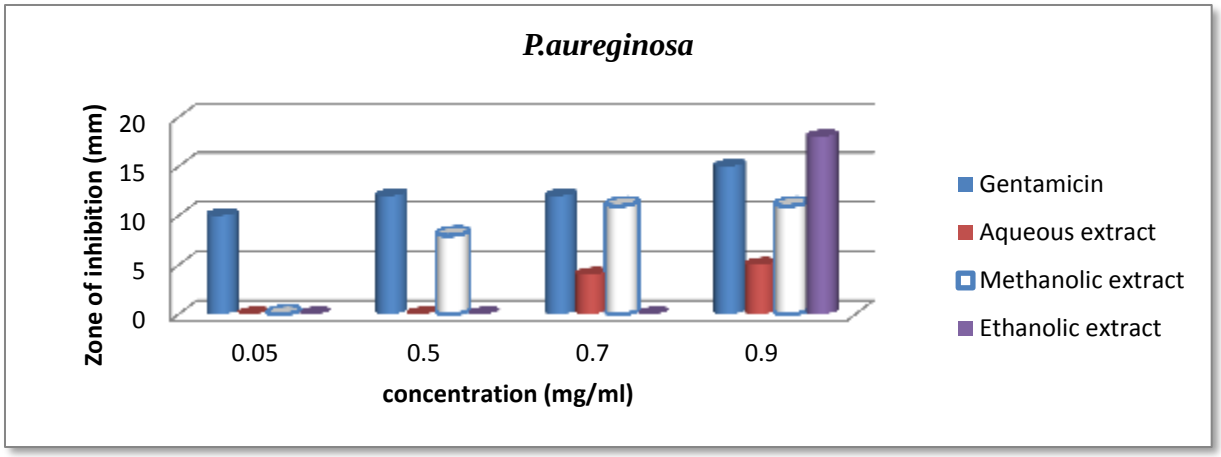

Figure-5 Graphical representation of anti bacterial activity of *Dolicus lablab* leaf extract against *P.aeruginosa.*

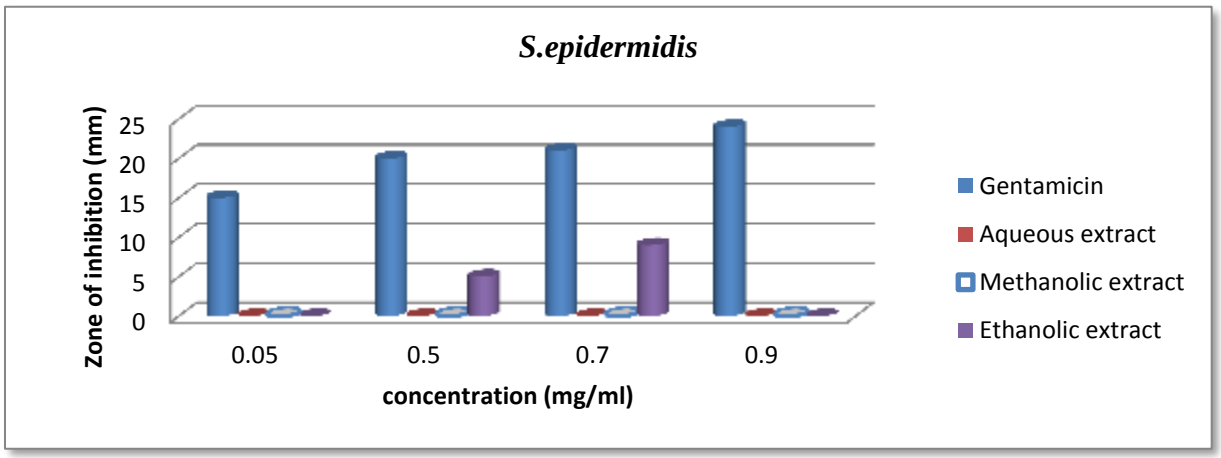

Figure-5 Graphical representation of anti bacterial activity of *Dolicus lablab* leaf extract against *S.epidermidis.*

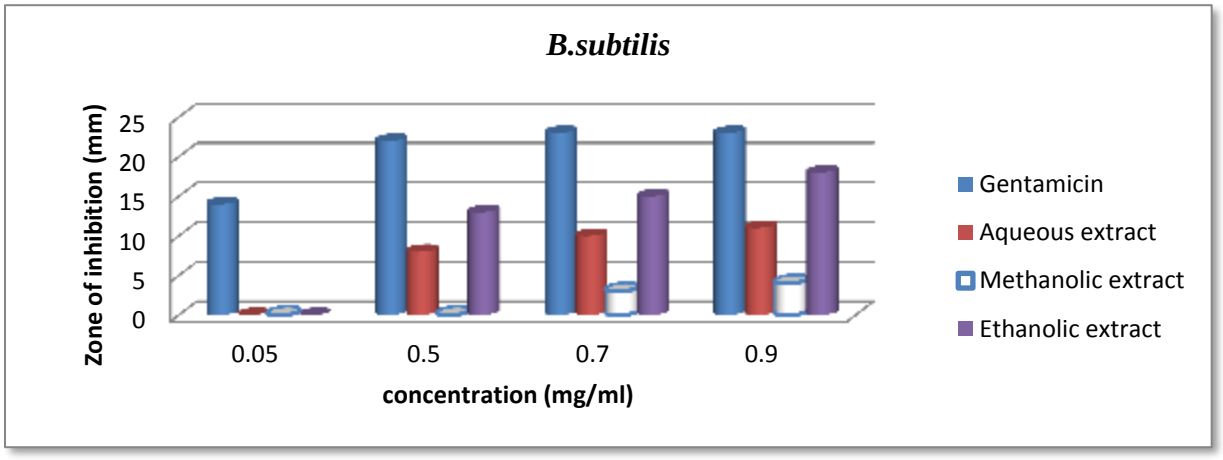

Figure-5 Graphical representation of anti bacterial activity of *Dolicus lablab* leaf extract against *B.subtilis*

From the table-1 it was evident that the flavonoids and saponins are present in all the extracts, alkaloids and terpenoids are present only in Methanolic extract and phenols are present in ethanolic and aqueous extracts. Good antifungal activity was exhibited by all the three leaf extracts of *Dolicus lablab* against all the three pathogenic fungal strains when compared with the standard drug. The results from table-2 says that among the three extracts methanolic extract showed significantly high zone of inhibition at a range of 15-24 mm against *Trichophyton rubrum*, 14-30 mm against *Trichophyton mentagrophytes* and 16-24 mm against *Candida albicans*, where as the ethanolic extract exhibited a moderate antifungal activity when compared to other two extracts against Trichophyton rubrum (10-17mm), Trichophyton mentagrophytes (13-19mm), Candida albicans(13-20mm) and the aqueous extract also reported a good result with a zone of inhibition of 16-21mm against T.rubrum,13-24mm against T.mentagrophytes and 17-22 for C.albicans. From the table-3, it was evident that the all the three extracts exhibited moderate to low antibacterial activity when compared with the standard. Aqueous extract has moderate potentiality to inhibit the growth of only one strain of bacteria ie., *B.subtilis* at average of 8-10mm, where as methanolic extract showed a good antibacterial activity against two Gram(-) bacteria, *E.coli*, *P.aureginosa*, with a Zone of inhibition at a range 15-19mm and 8-11mm at the selected concentrations, while ethanolic extract reported zone of inhibition against *E.coli* as 12mm, *S.epidermidis* as 13mm and *B.subtilis* as 18mm at the maximum concentration. *P.aureginosa* showed no zone of inhibition to ethanolic leaf extract and *S.epidermidis* didn't respond to aqueous and Methanolic extracts of *Dolicus lablab* leaf extracts and reported nill zone of inhibition. We have tested the MIC values for the extracts showed excellent or good antimicrobial activity. The result of MIC observed for

methanolic extract against E.coli is at 40 µg/ml, T. rubrum is at 60 µg/ml and T.mentagrophytes at 60 µg/ml.

CONCLUSION

From the present study, it was observed that the methanolic leaf extract of *Dolichos lablab* was found to have a significant antimicrobial activity. The results of the study says that the leaf extract is potential to fight against pathogenic fungi than bacteria. The phytochemical analysis of *Dolichos lablab* shows the presence of different secondary metabolites or phytochemicals such as alkaloids, flavonoids, glycosides, phenols, saponins & sterols. The antimicrobial activity exhibited by *Dolichos lablab* may be attributed to these phytochemicals in it. Therefore the result of present study showed that *Dolichos lablab* as an antimicrobial agent. However further pharmacological studies are required to be undertaken to understand the underlying possible mechanisms of the observed activities as well as need to isolate, purify, Characterize active phytochemicals responsible for these bioactivities.

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