World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

EVALUATION OF ANTIMICROBIAL AND WOUND HEALING ACTIVITY OF *LABLAB PURPUREU*

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Article Received on 26/03/2020 **Article Revised on 16/04/2020** **Article Accepted on 06/05/2020**

ABSTRACT

For the current study medicinal plant *Lablab purpureus* was selected. The introduction of antibiotics transformed the way by which infectious sickness were treated. Rapidly, widespread infections became effortlessly curable and outbursts of infectious ailment were eagerly controlled. Though, the announcement of triumph over bacterial pathogens was early. Antimicrobial resistance rapidly emerged to decrease the clinical value of every novel antibiotic that was developed. Alleviation of antimicrobial resistance is therefore essential, and necessitates that veterinarians and further health proficient's appreciate antibiotic sensitivity and resistance at the organism, population, molecular and cellular levels. The effort is intended for the antimicrobial action for (bacterial and fungal species) and separation and categorization by spectral analysis. Followed by antioxidant and wound healing evaluation of the extracts and spectral analysis for isolation and characterization of active compounds. The research presented in this thesis focused on antimicrobial, antioxidant and wound healing action of such resource, as plants are recognized to be fairly resistant to most microbes are traditionally used for ailments like wounds, burns etc. Therefore, specific focus was given on antimicrobial activity, antioxidant and wound healing activity.

KEYWORDS: Antimicrobial, Wound Healing Activity, *Lablab purpureu*, Antioxidant activity.

INTRODUCTION

Natural drugs are characterized as a division of science in which herbal or plants or parts of plants are utilized to ease from various ailments. It may also be called as herbal drugs or phytomedicines. Of late phytotherapy has been presented as more precise equivalent word of natural or plant prescription. In the mid-twentieth century, the natural drug was the structure of the main medical services, since no analgesics or uninfected agents were found. With the allopathic disposition of the pharmacological approach, the natural prescription has constantly lost its reputation among individuals, which depends on the rapid aid activities of synthetic drugs.[1] Plants are a fundamental piece of nature. Physical excellence and its science are of enormous importance to humanity. They are the life-supporting power on earth. Jethro Kloss, a naturopath, says that there is a magnificent science in nature, in trees, herbs, roots and blooms which man has never comprehended. He additionally expresses that if genuine cures are found in nature, at that point noxious Herbal medicine or recipe grown at home is seen as the use of herbs to be treated disorder or the use of herbal plants for their regenerative and therapeutic esteem.[2] It incorporates Traditional Chinese medication, Chiropractic, Biofeedback, Unani, Ayurveda, Meditation, Homeopathy, Yoga, Hypnosis, Naturopathy and Acupuncture. Generally, numerous herbs have been utilized to give human solutions for diseases that face men and ladies. The restorative plants expect a colossal significance, particularly when the world is demonstrating a resurgence of enthusiasm for the mending properties. The world is supplied with a rich abundance of therapeutic plants. Herbs have dependably been the primary type of drug in India. Restorative plants are financially critical as they give the fundamental crude materials to pharmaceuticals, phyto-compound enterprises, medications and chemicals would be disposed of an affliction would be uncommon.

MATERIALS AND METHOD

Plant material: based on the traditional uses by traditional healer and other reported information, the plant was selected for the present study and embodied in the thesis. The plant materials used was seed of *Lablab Purpureus*.

Collection, identification and authentication of plant material: The plant material used in this investigation was seed of *Lablab Purpureus*; the seed was purchased from local market Indore, Madhya Pradesh, India was validated by the Botany Department, Govt. Holkar College, Indore (M.P). The shadow dried plant materials

were roughly powdered by a mechanical grinder and kept in a nylon bag inside a deep freezer, till further use.[3]

Physio-chemical characterization

Quantitative determination

The procedures suggested in **Indian pharmacopoeia** (Anonymous, 1985) and WHO procedure (1998) were followed to determine the water-soluble ash, acid-insoluble ash and total ash. The percentages of methanol soluble and water-soluble extractives were also determined.

Extractive value: Extractive values of the test materials were determined using 90% methanol and water.

Methanol soluble extractive method: Air dried 5 gm of macerated and roughly powdered sample was soaked in 100 ml of 90% methanol in a covered flask for 24 hours with frequent trembling after an interval of 6 hours and permitted to rest for 18 hours. After rapid filtration, some precautions have been taken to avoid solvent stirring. The approximately 25 ml of filtrate was concentrated to dryness, dried at 105 ° C and weighed. The proportions of the extractive values were considered with respect to the reference of the air-dried sample.[4]

Water-soluble extractive method Coarsely powdered 5 gm of air-dried macerated sample was flooded with 100 ml of chloroform water (95 ml of water + 5 ml of chloroform) in a covered flask for overnight, with frequent shaking after an interval of 6 hours and permitted to rest for 18 hours, followed by rapid filtration. The filtrate about 25 ml was concentrated to dryness at 105°C and weighed. The proportions of the extraction values were calculated with respect to the reference of the air-dried sample.[5]

Ash Value: The remains left subsequent to incineration are known as ash content of crude drug. It generally signifies the naturally occurring inorganic salts in the drug and stick to it, but it may also contain inorganic substance supplemented for the reason of adulteration. There is a significant variation in the ash content of various drugs, but the variation varies with fine limits.

Total ash determination: Precisely weighed 2 gm of the thickly powdered sample was kept in a crucible and air- dried; it was earlier ignited and cooled prior to weighing, at a temperature up to 450°C. The ignition was estranged till constant weight was obtained. Compared to reference the proportion of ash of air-dried sample was calculated.[6]

Determination of water-soluble ash: After the determination of total ash, the ash obtained was subjected to boiling for about 5 minutes with addition of 25 ml water and ash less filter paper was used for filtration. The filtrate was further washed with aid of hot water for 15 minutes and then ignited at a temperature below 450°C; simultaneously it was cooled and weighed. The insoluble matter weight is subtracted from the weight of total ash. The variation in weight was measured as water-soluble ash concentration.[7]

Determination of acid insoluble ash: After the determination of total ash, the ash obtained was subjected to boiling with 50% HCL for nearly 5 minutes. With help of filter paper, the insoluble ash was collected and cleaned by hot water, then ignited, cooled and weighed. The methodology was rehashed to get the consistent weight. In comparison to reference the percentage of acid-insoluble ash was calculated of air-dried sample.

Sulphated ash: In a crucible 2 g of the air-dried coarsely powdered sample were carefully taken. Then 4-5 drops of conc. sulfuric acid was added and ignited at a temperature up to 450 ° C for at least 3 hours in a hot air oven and then cooled. Then again 4-5 drops of conc. H_2SO_4 was added and turned on, then cooled and weighed. In comparison with the reference, the percentage of sulphated ash from the air-dried sample was calculated.[8]

Extract Preparation: The plant resources were primarily washed with distilled water and dried using paper towel in laboratory for 24 h at room temperature and pulverized into powder (coarse) by a mechanical grinder. Accurately weighed 1 Kg of the plant resources was firstly defatted with petroleum ether and were extracted with benzene, chloroform, ethyl acetate, ethanol and aqueous solvents in a soxhlet extractor. The percentage yield of petroleum ether, alcoholic, chloroform, ethyl acetate, benzene and aqueous extract were found to be 8 gm, 12 gm, 15 gm, 18 gm, 18 gm and 20 gm, respectively. The standard extracts obtained from *Lablab Purpureus* were packed in an air tight container and then stored in the refrigerator at a temperature of 4 ° C for use in phytochemical detection. Following the results of antimicrobial activity, the ethyl acetate dry extract was stored in a vacuum dessicator and subjected to phytochemical analysis.,[9,10]

***In-Vitro* Antimicrobial Activity**

Microbial strains Extracts of *Lablab Purpureus* was tested against the following five microbial strains.**[11,12]**

- *Escherichia coli*
- *Staphylococcus aureus*
- *Pseudomonas aeruginosa*
- *Bacillus Subtilis*

Agar Well Diffusion assay: Hi-media agar plates were arranged and 6mm wells were made and wiped with aforesaid cultures and all the wells were then added with standard and plant extracts individually and the prepared plates were incubated at 37°C for overnight.[14]

Microbiological media used for Bacteria: The media for different bacterial strains was: Hi-media Nutrient

agar, Composition (G/Litre): Beef extract 10.0; Sodium chloride, 5.0; Peptone 10.0 (pH 7.2).[15]

Microbiological media for Fungi: For fungal growth media used was Potato dextrose agar (Himedia), Composition (G/Litre): Dextrose 20.0, Potatoes infusion 200.0 (pH 5.2).[16]

Maintenance of Microbial strains: Sterilization of media was done by autoclaving at 121°C temperature and 15 lbs pressure for at least 15 minutes. They were poured in glass petri plates and keep aside for solidification. After solidification the plates were dried in an incubator for 30 minutes to remove the excess of moisture from the surface. Most of the pathogenic bacteria are susceptible to antibiotics or other antimicrobial agents but their response varies enormously. The susceptibility is based on genetically determined characteristics of individual species when antimicrobial agents are used to treat infectious, resistant strains of originally sensitive organism have developed. The nutrient broths were added in the test tubes plugged with cotton and autoclaved. Label the test tubes as per the types of the bacterial cultures. The nutrient broth was inoculated with given bacterial strains and was incubated at 37 ± 1°C for 24 hours. The test microbial strains were inoculated after incubation period using sterile cotton swab in the nutrient agar plates. For the Antifungal activity Potato dextrose agar (Himedia) was used. In the Potato dextrose agar (Himedia) plates, the fungal strain *Aspergillus niger* was inoculated. The inoculated plates were placed for incubation at 37°C ± 1°C temperature for at least 48Hour.[17,18]

Concentration of the Extracts/Fractions taken was as: Each sample extracts (200mg) were suspended in particular solvents (1 ml). Hi-media antibiotics: Amphotericin-B (100 units), Streptomycin (10 microgram) were used as standards. The bacterial strains count and fungal strain count was limited to yield 1 X 10^7 to 1 X 10^8 mL^{-1} and 1 X 10^5 to 1 X 10^6 mL^{-1} respectively. The microbes (0.1 ml) were inoculated using a sterile spreader over the surface of nutrient agar media plates. The agar plates inoculated with test organism were incubated for one hour prior placing the extract in the wells of agar plates. In BOD incubator all bacterial plates were kept at 37 ± 0.1°C for overnight. After incubation period every plate was seen for zones of inhibition and by help of a vernier calliper the diameters of these zones were calculated in millimeters. Sterile conditions were maintained for all tests performed and all tests were performed in triplicate. Amphotericin B (100 unit/well) and Streptomycin (10µg/well) were used as positive controls.

Antioxidant and Wound Healing Activity Of *Lablab Purpureus:* Wound healing procedures are efficient biochemical and cell occasions prompting the development and recovery of wounded tissue in an uncommon way. Healing of wounds is a vital organic process including tissue repairs and recovery. It includes the movement of a perplexing system of platelets, cytokines, and development factors which at last prompts the rebuilding to ordinary state of the harmed skin or tissue. The goal of wound care is to advance wound healing as quickly as possible, with less pain, distress and scarring in the patient, and should occur in a physiological domain useful for tissue repair and recovery. Wound healing procedures are known to be affected by different factors such as by contaminations, nourishing status, medications and hormones, sort and sites of wound, and other ailments like diabetes.[19]

In *vitro* antioxidant activity: Antioxidant activity not supposed to be reported on the basis of solo antioxidant analysis report. Many *in vitro* test processes are performed for estimation of antioxidant activity. Another characteristic is that antioxidant analysis models differ in diverse respects. So, it is not sensible to evaluate one method over another. Usually *in vitro antioxidant* model by free radical traps are comparatively simple to perform. Amongst free radical scavenging technique, DPPH technique is simple, quick and cheap in comparison Superoxide radical scavenging activity and α-Amylase inhibitory activity.[20]

DPPH scavenging activity: Principle: Free radical scavenging property of the extracts/fractions were evaluated (*in vitro*) against the methanolic solution DPPH (α,α-diphenyl-β-picryl hydrazyl). Generally the antioxidants combines with DPPH and change it to α,α-diphenyl- β-picryl hydrazine (Fig. 5.1). The DPPH is stable free radical, purple in color and upon reaction with an antioxidant; it becomes colorless and the extent of discoloration point toward the scavenging activity of the antioxidant extract. The variation in the absorbance created at 517nm has been used as a measure of antioxidant activity.

The IC_{50} value is known as the quantity of the sample enough to elicit 50% reduction of the primary DPPH concentration, was considered from the linear regression of the test concentration graphs to the average percentage of antioxidant action got from the three replicate tests. Also the free radical scavenging activity of ascorbic acid (Vitamin C) was also calculated under the similar situation to serve as +ve control.[21,22]

Method: The molecule α,α-diphenyl-β-picrylhydrazyl (1, 1-diphenyl-2-picrylhydrazyl; DPPH) is differentiated by the delocalization of the extra electron above the molecule as an intact, so that the molecule does not dimerize. The delocalization of electron results in formation of deep violet color, illustrated by an absorption band in ethanolic solution at nearly 517 nm. When DPPH solution is added to substrate that it can contribute a hydrogen atom, then this will give result to the reduced form with the reduction of violet color. Sequentially to assess the antioxidant activity by eliminating free radicals from the samples, the variation

in the optical density of the DPPH radicals is observed. The test extracts and fractions in various concentrations (0.2 ml) are diluted by methanol and 2 ml of DPPH solution (0.5 mM) is mixed. At 517 nm the absorbance is calculated after 30 minutes. The proportion of the DPPH radical scavenging is determined by using the formula as given beneath:

$$\text{DPPH radical scavenging activity (\%)} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \cdot 100,$$

Superoxide Radical Scavenging Activity Method

Principle: Superoxide radical is recognized to be very dangerous to the cellular mechanisms. The evaluation was based on the ability of sample to hinder farmazan construction by scavenging the superoxide radicals produced in riboflavin-light- nitro blue tetrazolium (NBT) system.[26]

Alpha-Amylase Inhibitory Activity: The α-amylase action was calculated by the dinitrosalicylic acid (DNS) method proposed and accepted for testing α-amylase inhibitory potential by means of 1% soluble starch as substrate. The test substance was pre-incubated by amylase (100 µL) on room temperature for 20 min previous to the adding up of 100 µL of the substrate solution after that incubation at 37°C for 10 min.[27]

The reactions were closed by adding up of 200 µL of DNS reagent after that color growth by introduction of the tubes in boiling water for 5 min and then added 3.6 mL of distilled water. Acarbose was considered as positive control. The absorbance was taken at 470 nm and experiments were carried out in duplicates.[29]

Wound Healing Activity

Wound ointment preparation

The dry plant extracts were used to prepare the ointment. Simple Ointment (British Pharmacopoeia) base was prepared as follows: white soft paraffin, cetostearyl alcohol, hard paraffin and lanolin were weighed in a crucible in 17:1:1:1 ratio. The crucible was placed in a water bath at 85°C to melt the contents, after which these were stirred constantly until the mixture solidified. The 10% and the 20% *Lablab Purpureus* ointments were prepared by triturating 10 grams and 20 grams of various extracts respectively into 90 grams and 80 grams of the earlier formulated simple ointment.The trituration was done on a glass slab using a stainless steel spatula until a uniformly mixed ointment was achieved. The respective ointments were dispensed into separate labeled containers and stored at room temperature awaiting use. The above mentioned simple ointment (without plant extracts) was used on control groups.[30]

Determination of LD_{50}: Herbal medications are frequently regarded as safe because of their natural origin. Though, these foodstuffs have bioactive constituents with the probability of leading undesirable effects. The reason of this acute toxicity study is to decide the nature and amount of the problematical reaction that may go after the intake of a solo dose of the drug. A quantitative characteristic of the acute toxicity study is the measurement of the fatal dosage of the test samples. The acute toxicity test facilitates in the preparation of the dosage level in animal researches. The LD_{50} of *Lablab Purpureus* was anticipated by subsequent up and down stair case technique in animal. Dosages were accustomed by a constant multiplication factor (viz., 4) for this study. The dosage for every consecutive animal was used to depending upon the preceding outcomes. The gross effect and acute toxicity of crude extract of *Lablab Purpureus* was calculated in albino rats by means of ½ LD_{50} dose. A whole of six animals were chosen for every experiment. Animals were seen at hourly intermission for 6 hours and over again after 24 h. the limitation for gross effect and motor activity were calculated following administration of *I Lablab Purpureus* orally at dosage level of 2.5 g/kg of the body weight. Alterations in fur and skin, mucous membranes and eyes, and behavior pattern and respiratory rhythms were main guidelines of examination. Particular attention was provided for interpretation of convulsions, tremors, diarrhea, salivation, sleep, lethargy and coma. Alterations like food and water intake, body weight variations were traced at two days gap. There was no morbidity or mortality seen in animals during the 14-day phase followed by solo oral dose administration at all chosen dosage levels of the ethyl acetate extract of *Lablab Purpureus*. Morphological characters (fur, eyes, skin and nose) were regular. No signs of convulsion, tremors, diarrhoea, salivation, lethargy, or strange behaviors for instance walking backward, self mutilation were observed; posture and gait, sensitivity to handling or sensory stimuli, and grip strength were all usual.[31]

Acute dermal toxicity study: For dermal toxicity, two groups a total of 6 animals in each group were used. Animals having normal skin surface were kept separately in a cage and made accustomed to the laboratory circumstances for a period of five days past to the test. Near about 10 % of the total surface area of body were shaved from dorsal part prior 24 h of the study. Then extract ointments in concentrations (10% and 20%) were used on the clean shaved area. At the end of the exposure phase (24 h), the remaining test substance was detached and the animals were kept under observation for 24 h and subsequently for 14 days animals were seen for intensification of any unfavorable skin responses like irritation, inflammation or redness. Additionally, skin toxicity of extract ointments was measured when the ointment was used for several days (> 10 days).[31;32]

Dosing and Grouping of animals For the excision & incision model seven groups of animals, each consisting six animals were used. The primary group was applied with simple ointment which acted as a negative control. Group II acted as standard, which was treated by framycetin sulfate cream (Soframycin, Aventis)

0.2%w/w. Group III was applied with Benzene extract (10% w/w) and Group IV applied with chloroform extract (10% w/w), Group V applied with ethyl acetate extract (10% w/w), Group VI applied with Ethanolic extract (10% w/w) and Group VII applied with Aqueous extract (10% w/w) of *Lablab Purpureus.* Members of respective groups were marked appropriately with two sets of marks on each animal's tail, one depicting the treatment group the animal belonged to and the other designating the individual animal number within the group, as revealed.

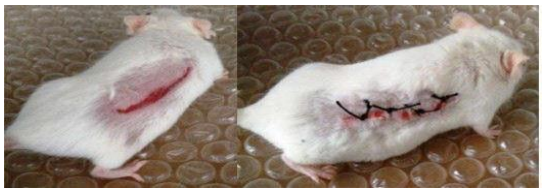
Wound healing activity: For the present study Albino rats (Wistar strain) of either sex with 150-250 g weight were used. The inbred animals were made used to the experimental situation in the animal house of the organization. The animal house was finely kept in average hygienic circumstances, at 22±2ºC temperature, 60%±10% room humidity through 12 h day and night cycle, with food and water *ad labium*. They were offered by purified water and marketable food pellets. Animal preservation was as per the CPCSEA (Committee for the purpose of Control and Supervision of Experimentation of Animals) guidelines and every experiment on animals were performed as per the internationally established principles for laboratory animal usage and as per the experimental procedure duly permitted by the Institutional Ethical Committee (IAEC No. SBRL/IAEC/PN-18039).

Excision wounds

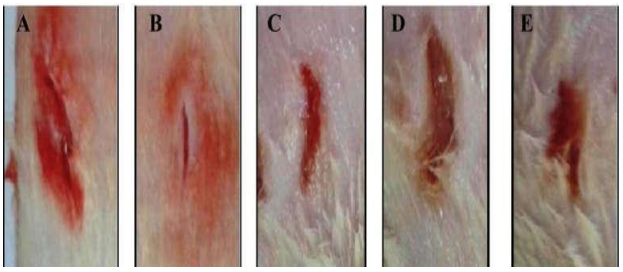
The animals were at random separated into 7 groups, each containing of 6 animals. Group I was recognized as untreated control and simple ointment was applied over it. Group II was considered as the standard group, Framycetin sulfate cream (Soframycin, Aventis) 0.2% w/w was applied over it. Group III was applied with the Benzene extract (10% w/w) and Group IV was applied with the chloroform extract (10% w/w), Group V was applied with the ethyl acetate extract (10% w/w), Group VI was applied with the Ethanolic extract (10% w/w) and Group VII was applied with the Aqueous extract (10% w/w) of *Lablab Purpureus*, till absolute epithelization. The animals were previously anesthetized for creating the wounds, by means of 1mL of intravenous ketamine hydrochloride (10mg/kg). As explained by Morton and Malone the rats were inflicted with excision wounds. A mark was created on the dorsal thoracic area 5 cm away from ear and 1 cm away from vertebral column on the anaesthetized animal. An electric clipper was used to shave the dorsal fur of the animals and the area to be wounded was marked on the posterior of the animals. A complete thickness of the excision wound of spherical area of 500mm^2 and 2mm deepness was made by means of scalpel, toothed forceps and pointed scissors. Haemostasis was achieved by blotting the wound by cotton swab flooded in normal saline. The complete wound was kept open. Every surgical event was performed in aseptic conditions. The medicaments were topically used one time daily, starting from the first day. The wound area was measured on 4th, 6th, 8th, 11th, 14th day and 16th day subsequent to wounding. This was accomplished by tracing the wound area on a graph paper. Decrease in the wound area was shown as percentage of the initial wound size. The same procedure was repeated for various fractions obtained from ethyl acetate extract.

Measurement of epithelization time: The epithelization period was considered as the number of days needed for declining off of the dead tissue remaining with no any outstanding raw wound.

Incision wounds: The rats were at random separated into 7 groups, every group having 6 animals. Group I act as control (untreated) and was treated with simple ointment. Group II was considered as standard group was applied with framycetin sulfate cream (Soframycin, Aventis). Group III was applied with Benzene extract (10% w/v) and chloroform extract10% w/v) was applied to Group IV, Group V was applied with ethyl acetate extract (10% w/v), Group VI was applied with Ethanolic extract (10% w/v) and Group VII was applied with aqueous extract (10% w/v) of *Lablab Purpureus*. The animal's dorsal fur was detached by a depilator cream before the wounding. A longitudinal para-vertebral incision 2 mm deep and 6 cm long was created by a sterile scalpel throughout the cutaneous muscle and skin on the dorsal surface. The wounds were blocked with surgical sutures at a gap of 1 cm. Topically the extracts were applied one time daily, starting from the first day for 10 days. The sutures were taken out on the 8th day and the tensile strength was measured of the healed wound on the 10th day, with the 'Continuous Water Pouring Technique' (Lee *et al.*, 1970).[34]



Images of Incision Wound Model.



Images of Incision Wound Model Day (A) 02 day, (B) 04 days, (C) 06days, (D) 08days, (E) 10 days.

Measurement of tensile strength: The animals were anesthetized and moved to the operating table. Two forceps were tightly utilized 3 mm far from the limit of wound confronting each other on inverse side of the cut injury. One of the forceps was settled on stands, while

the other was associated with an openly suspended lightweight plastic of volume 1000 ml through a string keep running over to a pulley. Water was permitted to stream persistently from the repository gradually and relentlessly into the holder. The minute the injury simply opened up the water stream was captured and the volume of water gathered in the holder (roughly equivalent to its weight) was noted as tensile strength [35].

TS (untreated)
% Tensile strength (TS) of Control = TS (control) – TS (untreated) X 100
TS (Control)
Tensile strength (TS) of Standard = TS (Standard) – TS (Control) X 100
TS (Control)
% Tensile strength (TS) of Test sample = TS (extract) – TS (Control) X 100

Illustration Tensile Strength Determination

Excision Wound Model (Infected Wounds): The outcome of wound healing models made it obvious that the ethyl acetate extract of *Lablab Purpureus* have moderately superior wound healing activity over other extracts. So, wounds were contaminated by *Staphylococcus Aureus* and *Pseudomonas Aeruginosa* and separate study was carried out with ethyl acetate extract on this infected wound model. The chosen animals were separated into three groups, every group containing 6 animals. A circular seal of 20mm diameter was created on the either sides of the central trunk. Excision wound was exacted on the rats as portrayed before. An excision of full skin thickness was made to get a wound measuring around 314mm^2.[36]

Determination of wound microbial load: Using sterile swab sticks at 4th, 8th and 16th days of post-treatment (dpt) wound mops from every animal was collected in replica. The entire viable count was calculated subsequent to the standard plate counting technique. Temporarily, every mop was inoculated into the sterile nutrient broth to create stock solutions which was evaluated for double-fold (10^{-2}) dilutions. By means of a sterile pipette, 0.1 ml of the 10^{-2} dilution was brought on the exterior of sterile nutrient agar and the inoculums was evenly distributed by a sterile glass spreader. Further, the inoculated plates were kept for incubation at 37°C for overnight and colony counter was used to count the colonies.[63]

Histological Examination: The histopathological study of the experiment was done on 16th day and the area of the neoepithelization of the wound was removed for histology. Five micrometer thick sections of the tissue were stained with haematoxylin and eosin. The tissue samples were evaluated for certain histological criteria. The different animal groups were reviewed frequently and outcomes were evaluated with the control groups.[37]

Statistical analysis The facts acquired in the investigation were processed by ANOVA (one way of analysis of variance) for decisive the considerable variation. The Dunnet's *t*-test was used to analyze the inter group significance. The P value <0.005 is considered as significant. All the values are expressed as Mean ± SEM.[38]

RESULT AND DISCUSSION

Quantitative Determination: The ash value of a crude drug is usually the remainders left subsequent to incineration. It generally signifies the inorganic salts occurring naturally in the drug and adhere to it, but can also include inorganic substance added for the reason of adulteration. There is a significant distinction varies within fine limits in context of the similar entity drug. So ash value determination provides a basis to evaluate the character and purity of a drug and provide data related to adulteration with inorganic material. For a number of official drugs, ash standards have been established. Typically these standards get a highest limit on the overall ash or on the acid insoluble ash acceptable.

Table 2: Different Ash Values of *Lablab Purpureus.*

ASH VALUES	Values in %
Total ash	9.0
Acid insoluble ash value (dil.Hcl)	3.6
Sulphated ash value (H_2SO_4)	20.8
Water soluble ash value (H_2O)	8.5

Extractive Values: Extractive values are helpful for assessment of crude drugs and provide a design about the chemical nature and constituents present. A drug yields fixed amount of extractive under a certain solvent is frequently a fairly accurate measure of a definite constituents or groups of correlated constituents the drug might contain.

Table 3: Different Extractive Values of *Lablab Purpureus.*

Extractive value	Values in % w/w
Alcohol soluble extraction	1.5
Water soluble extraction	1.7

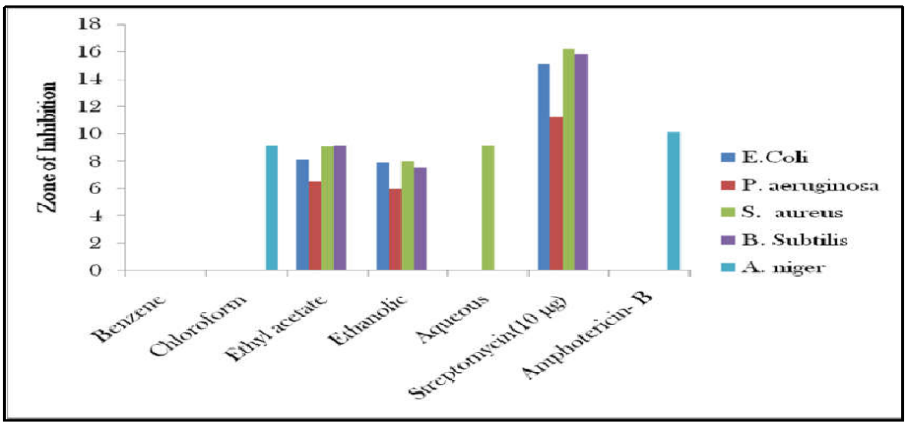
Preliminary phytochemical study of extracts of *Lablab Purpureus:* Due to variety of phytochemical constituents present inside the medicinal plants they act as natural healers in addition to this they also cure various human ailments.

Antimicrobial assay of Extracts: The antimicrobial activity of five specific extracts of *Lablab Purpureus* is tabulated in Table in the terms of zone of inhibition. DMSO was used as control and it didn't show any signs of inhibition against all microorganisms used for the study. All the extracts except chloroform failed to inhibit growth of *A. Niger*. Ethyl acetate extract inhibited almost all tested gram-positive, gram-negative bacteria with zone of inhibition 8.13mm against *E. coli*, 6.53mm against *P. Aeruginosa*, 9.12 mm against *S. Aureus*, and 9.15mm against *B. Subtilis* but showed no effect on fungi *A. Niger*.

Table 4: Antimicrobial Activity of *Lablab Purpureus* Fractions.

Fractions/ Standards	Zone of inhibition (mm)				
	Gram Negative bacteria		Gram positive bacteria		Fungi
	E.coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*A. niger*
Benzene (IPR 1)	6.21	6.72	6.03	5.29	-
Chloroform (IPR 2)	6.04	5.21	5.63	6.14	8.03
Ethyl acetate (IPR 3)	9.57	8.40	11.02	11.27	-
Ethanolic (IPR 4)	6.31	6.58	6.97	6.41	8.21
Aqueous (IPR 5)	5.14	5.76	8.74	8.19	-
Streptomycin(10 µg)	15.11	11.23	16.23	15.78	NA
Amphotericin- B	NA	NA	NA	NA	10.11

Diameter of zones in mm determined by Vernier Caliper;
'-' means no zone of inhibition, NA: Not applicable.
* Readings below 5 mm were not considered.



Graphical Rep resentation of Antimicrobial Activity Shown by Different

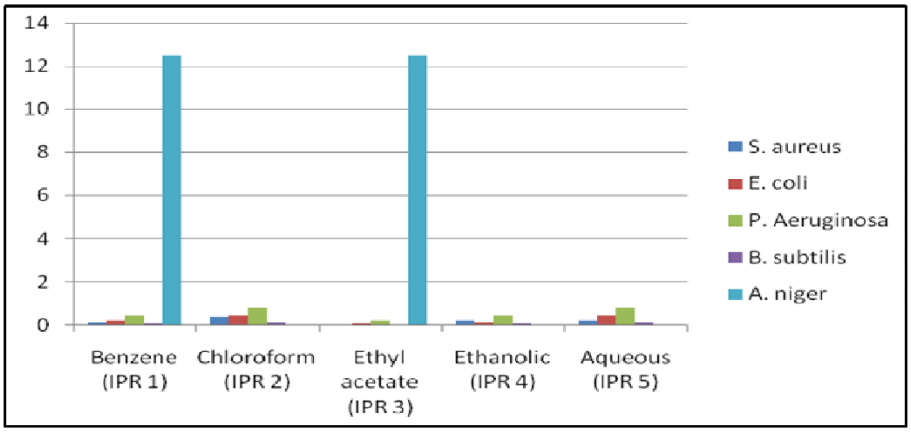
Diameter of zones in mm calculated by Vernier Caliper; '-' means no zone of inhibition, NA:Not applicable. * Readings below 5 mm were not considered.

MIC studies of Extract s of *Lablab Purpureus:* Minimum inhibitory concentration (MIC) in microbiology is defined as the minimum concentration of an antimicrobial (like an antibiotic, bacteriostatic or antifungal) agent that will restrict the evident increase of a microorganism after 24 hours incu bation period. With the help of agar plates or broth dilution methods (in liquid growth m edia) MIC can be calculated.

Figure 5: MIC Studies of Various Extracts of *Lablab Purpureus.*

Extracts		MIC values (in mg/ml) against			
	S. aureus	*E. coli*	*P. aeruginosa*	*B. subtilis*	*A. niger*
Benzene	0.39	0.39	0.78	1.56	NA
Chloroform	0.78	1.56	3.12	0.39	NA
Ethyl acetate	0.10	0.20	0.39	0.10	NA
Ethanolic	0.39	0.39	1.56	0.20	NA
Aqueous	1.56	1.56	3.12	0.39	NA

Minimum Inhibitory Concentration (rounded to two decimal places) of *Lablab Purpureus solvent* extracts against the tes ted microbes. Key: NA = No activity.



***IPR-1 (Benzene Extract), IPR-2 (Chloroform Extract), IPR-3 (Ethyl Acetate Extra ct), IPR-4 (Ethanolic Extract) and IPR-5 (Aqueous Extract)**

Graphical Representation of MIC of Different Fractions of Ethyl Acetate

Determination of *In-vitro* antioxidant activity

DPPH scavenging activity: The DPPH is stable free radical which reacts with appropriate reducing agent (hydrogen), to become paired off (diamagnetic molecules) and solution be converted into colorless stoichiometrically relying on the number of electron in use. The capabilities of all the extracts of Lablab *Purpureus to* scavenge DPPH were measured *in-vitro* the related IC50 values and the % scavenging results are mentioned in table. In a concentration dependent technique the test extracts scavenges the DPPH radical. The antioxidants combine with DPPH which leads to formation of purple colored steady free radical and change it into a monochrome (colorless) α-α-diphenyl-β-picryl hydrazine. The amount of DPPH reduced could be enumerated by calculating a reduction in absorbance at 517 nm. The IC_{50} value was found to be 9.87±0.54 $\mu g/ml^{-1}$, 23.65±1.98 $\mu g/ml^{-1}$, 36.41±1.54, 42.21±1.31 $\mu g/ml^{-1}$ and 58.26±0. Mg/ml-1 for ethanolic, Ethyl Acetate, Aqueous, Chloroform and Benzene extracts while the IC_{50} value of Vitamin C was 4.12±0.21µg/ml significantly reduced DPPH radical by bleaching it. As per the results, it may be suggested that the ethanolic extracts of *Lablab Purpureus* contains hydrogen contributors thus it is capable of scavenging the DPPH free radical.

Effect of extracts of *Lablab Purpureus in* Superoxide radical scavenging assay: ssdecline of absorbance at 560 nm by means of antioxidants points out the utilization of superoxide anion inside the reaction mixture. The data obtained clearly illustrates the percentage inhibition of superoxide radical creation by ethanol extract was highest among all the extracts used. The ethanol extract have powerful superoxide radical scavenging action and showed higher superoxide radical scavenging action which was similar as compared to the standard. In the superoxide radical scavenging technique, the ethanolic extract once more showed highest IC_{50} activity 96.66 $\mu g/mL^{-1}$ among all the samples used followed by benzene extract with a value of 88.01 $\mu g/mL^{-1}$.

Alpha-Amylase Inhibitory Activity: In the α-amylase inhibitory activity method, all the extracts exhibited potent antioxidant activity but ethanolic extract again was most potent among all 102.32 $\mu g/mL^{-1}$

Table 6: Effect of Various Extracts of *Lablab Purpureus* on IC_{50} Values by DPPH.

		IC_{50} ± SEM*(µg/mL^{-1})	
Extracts/ Standard	**DPPH**	**Superoxide radical scavenging**	**α-a mylase inhibitory activity**
Benzene	58.26±0.67*	88.01±0.59	135. 72±1.05
Chloroform	42.21±1.31	47.12±1.23*	192.25±1.54*
Ethyl Acetate	23.65±1.98*	52.11±1.28	352. 12±0.65
Ethanolic	9.87±0.54	96.66±1.41	102. 32±1.78*
Aqueous	36.41±1.54	87.42±0.89**	258. 21±1.52
Vitamin C	4.12±0.21		165±1.02
Acarbose	-	-	10. 4±1.25

Average of three independent determinations, three replicates, values are mean ± SEM. *Point out a significant differ ence at P < 0.005

**In the similar column with dissimilar superscripts differ significantly at P < 0.001

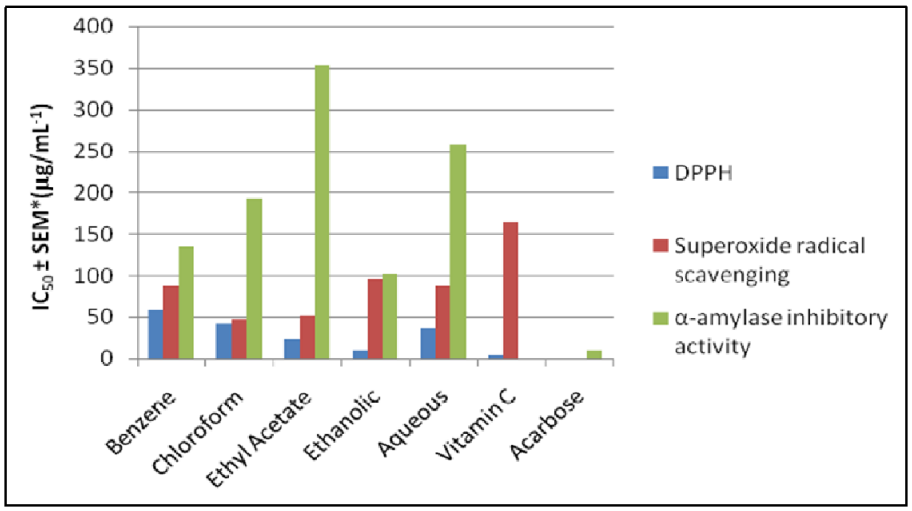


Figure: Graphical Repre sentation of All Extracts on IC_{50} Value by Different Models

Acute oral toxicity study of different extracts of *Lablab Purpureus:* For demonstration of the appropriate safe dose range a preliminary toxicity test was done for successive experiments rather than to give total toxicity information on the extract

Table 7: Acute Oral Toxicity Study of Different Extracts of *Lablab Purpureus.*

Extract	500 mg/kg extract	1000 mg/kg	1500 mg/kg extract	2000 mg/kg extract	3000 mg/kg extract	4000 mg/kg extract	Death	Death %
Benzene	Usual	Usual	Usual	Usual	Usual	Usual	00/06	0
Chloroform	Usual	Usual	Usual	Usual	Usual	Usual	00/06	0
Ethyl Acetate	Usual	Usual	Usual	Usual	Usual	Usual	00/06	0
Ethanolic	Usual	Usual	Usual	Usual	Usual	Usual	00/06	0
Aqueous	Usual	Usual	Usual	Usual	Usual	Usual	00/06	0

Acute dermal toxicity study: Following 24 h of appliance of the 10% and 20% ointment formulations, the location didn't illustrate any symbol of irritation, inflammation or redness. There was as well no obvious symbols and indications seen when the animals were observed for 48 hours. Furthermore, no symbols of toxicity and no transience were noticed throughout the 14 days cage side examination. But, the 20% extract ointment showed skin irritation and redness during successive application. However, the 10% extract did not show any irritation and redness even after several days of application and hence was chosen for the study.

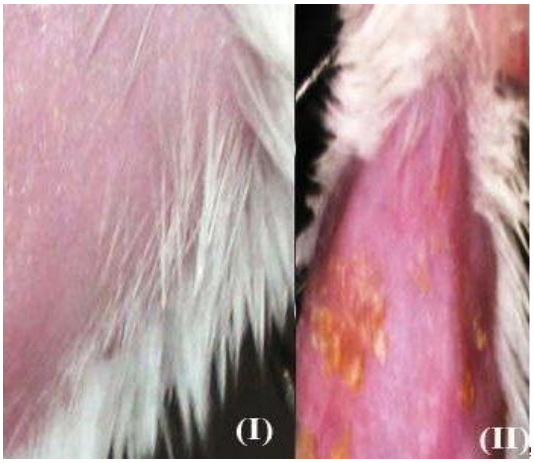


Image of Acute Dermal Toxicity Test Result.

(I): 10% w/w Ointment Application (II): 20% w/w Ointment Application

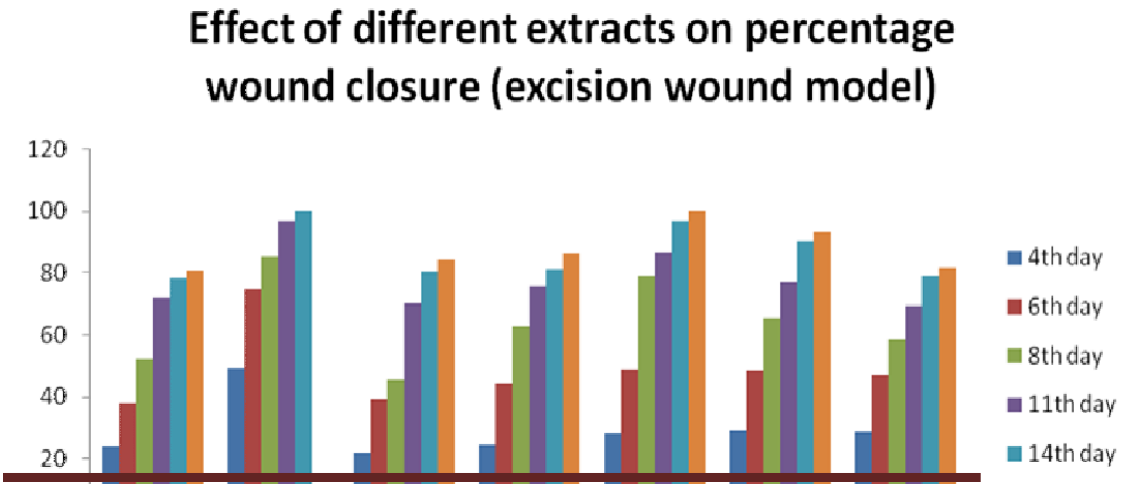
Wound healing activity: The outcome of the ex cision wound healing technique discovered that every group of animals treated with the plant extracts showed improved wound contraction constantly from 2nd day to 16th day or till th e day they were completely healed. The mean proportion of wound closing area was considered on the 4, 6, 8, 11, 14, and 16th po st wounding days. Every

reading is established t o be statistically important and similar with the control. The rats treated with ethyl acetate extract of *Lablab Purpureus revealed* the wound healing completed within 16 days as compared to other extracts of *Lablab Purpureus.* The epithelization period was found to be 16.43 ± 0.89**of ethyl acetate extract of *Lablab Purpure us relatively* which was comparable to Framycetin treated group (13.7 ± 1.52** days). Henc e the ethyl acetate extract of Lablab *Purpureus has* comparable effectiveness of action similar to the standard drug Framycetin in wound healing.

Wound healing activity

Excision wounds: The outcome of the excision wound healing technique discovered that every group of animals treated with the plant extracts showed improved wound contraction constantly from 2nd day to 16th day or till the day they were completely healed. The mean proportion of wound closing area was considered on the 4, 6, 8, 11, 14, and 16th po st wounding days. Every reading is established t o be statistically important and similar with the control. The rats treated with ethyl acetate extract of *Lablab Purpureus revealed* the wound healing completed within 16 days as compared to other extracts of *Lablab Purpureus.* The epithelization period was found to be 16.43 ± 0.89**of ethyl acetate extract of *Lablab Purpureus relatively* which was comparable to Framycetin treated group (13.7 ± 1.52** days). Hence the ethyl acetate extract of Lablab *Purpureus has* comparable effectiveness of action similar to the standard drug Framycetin in wound healing.

The effect of the extract on wound contraction is shown in T able. Wound area decreased with time and a significant difference ($p<0.01$) was observed between treatment and controls at all-time points with 10% extract ointment and standard. As observed from data, the ethyl acetate extract demonstrated major increase in proportion of excision wound closure. The percentage contraction was considerably more in ethyl acetate extract treated followed by ethanolic and chloroform extract treated in all days of contraction measurement.



Graphical Representation of Excision Wound Healing by Test Extracts Table Effect of Different Extracts of *Lablab Purpureus*.

Wound healing activity in infected wound: As we all are aware that the biggest risk with an open wound is that it is easily prone to microbial infection keeping it in mind, as ethyl acetate extract gave the best result in normal wound, so again the activity of ethyl acetate was tested against infected model by *S. aureus* and *P. aeruginosa*. The Wound healing action of the ethyl acetate extract of the *Lablab Purpureus* (excision wound model) inoculated by *S. aureus* showed epithelialization period of 19.41 ± 1.08 days as compared to standard (18.21 ± 1. 32) it showed a marked result. The Wound healing action exhibited by ethyl acetate extract of the *Lablab Purpureus* (excision wound model) inoculated by *P. aeruginosa* showed epithelialization period of 20.47 ± 0.69 days which is comparable to standard 18.93 ± 1.87days.

Table 8: Wound Healing Action of the Ethyl Acetate Extract of the *Lablab Purpureus* (Excision wound model) Inoculated by *P. Aeruginosa.*

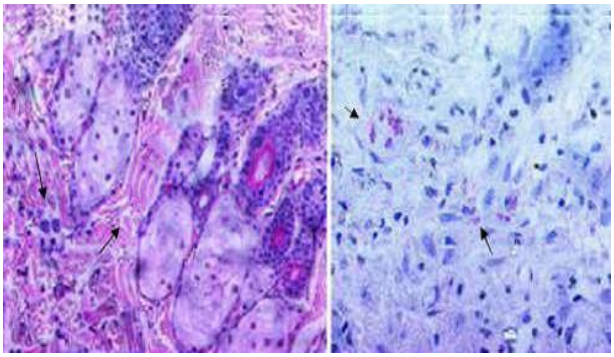
Group	Treatment	Percentage (%) wound closure						Time of epithelialization
		4th day	6th day	8th day	11th day	14th day	16th day	(Number of days)
I	Control	10.21±	16.94±	22.98±	33.77±	48.52±	52.54±	30.03 ±
		1.41	1.12	2.32	3.04	2.95	2.51	2.47
II	Framycetin	14.42±	26.13±	40.42±	58.13±	79.11±	92.09±	18.93±
		1.19	3.18*	2.38**	3.71**	2.84**	3.17**	1.87**
III	Ethyl Acetate	13.45±	22.4±	32.87±	50.2±	69.9 ±	83.91 ±	20.47±
	extract	1.27	2.12	2.07*	2.21**	3.57**	2.09**	0.69*

Bacterial load on the wound: On the 4th day post treatment, mean total viable count acquired in the groups treated by ethyl acetate extract (10 %) were considerably lower as compared against the control group. Alike movement was seen at 8th and 16th day post treatment.

Table 9: Total Viable Bacterial Cell Population from Excision Wounds of Rats.

Group (Treatment)	Mean ± standard error of mean total viable count at days post-treatment		
	4	8	16
Control	17.90 ± 2.78**	6.12±1.61**	1.41±0.32**
Framycetin	6.00 ± 1.49*	3.80±0.42*	0.70±0.27*
Ethyl acetate extract	6.30 ± 1.46*	4.25±2.28*	0.82±0.44*

Histological Examination: Healing was characterized by various histological changes representing the different phases of healing. On the 4th day post wounding, there were numerous neutrophils and intense neo vascularization with numerous endothelial cells in all the groups. In addition, there were few fibroblasts and macrophages, scanty collagen and a thin and incomplete epidermis at this time. Granulation tissue in wounds treated with Simple Ointment (B.P.) was loosely packed and interspersed with empty spaces which indicated the presence of edema; the few present fibroblasts had large elliptical nuclei and light chromatin density, showing that they were not yet fully mature. The granulation tissue in the 10% *Lablab Purpureus* ointment group was more densely packed than that in the simple ointment (B.P.) (control) group. The cells in the framycetin cream and the 10% *Lablab Purpureus ointment* treated groups were more mature with packed chromatin and intensely basophilic nuclei. On the 8th day of the wounds, there was a general reduction in the intensity of neo-vascularization and an increase in macrophage and fibroblast populations as well as collagen lay-down. Collagen deposition appeared to be more in the framycetin cream and in the 10% *Lablab Purpureus ointment* treated wounds. There was focal edema and congestion in sections from the framycetin cream treated wounds.On day 16, wounds from all the groups were approximately epithelialized. Vascularization and the number of neutrophils were diminished. There were numerous fibroblasts and more collagen but few macrophages. In the Simple Ointment (B.P.) group, the vascular response similar to that seen in the 8th day with numerous endothelial cells and capillaries was sustained and there was some edema in the granulation tissue. The granulation tissue also appeared to be more loosely deposited than that in the other groups. The 10% *Lablab Purpureus ointment* treated wounds had more collagen and a more compacted stroma. Moreover, the epidermis in the *Lablab Purpureus* ointments treated groups was denser than in the other groups with little spaces between individual cells.



DAY 4

DISCUSSION

A number of therapeutic herbs illustrated in Ayurveda still necessitate to be testified according to the contemporary parameters to make sure their action and efficiency. Therefore, *Lablab Purpureus* was selected on basis of its use in Ayurveda and conventional system of medicines. Many conventional health practitioners consider that the whole plant extract is more active than isolated compounds. In cases where full-grown trees or plants might not be found, the under grown suffice, which may result in accessibility of inconsistent plant material of the similar species. The amount of the

bioactive compound(s) from plants may vary with both the locality and the season in which they are collected. Moreover, the plants harvested from the wild usually differ in consistency and quality of dynamic compounds. Furthermore, the bioactive substance of many plants are poisonous enough if taken in excess and if the plant extract contains a lower than normal bioactive compound content, the sub-optimal dose may not be effective. Moreover, crude extracts from many medicinal plants may contain, in addition to the bioactive molecules, other constituents which have harmful effects.

Summary and conclusion: In the current past the medication world has adjusted their complete focus on the conventional drugs and natural treatment because of the consequences and toxicity of the man-made counterpart is gradually more. Therefore the current work was carried chiefly focused on recognition of antimicrobial ingredients from the conventionally used medicinal plants. Plants are the vital source of facts about modern medicine. Comparatively lower numbers of unfavourable reactions to plant products as for modern conventional pharmaceuticals, together with their cheap cost, is encouraging equally the consuming institutions for national health care and public to think plant medicines as an option to synthetic drugs. Currently herbal medicines are prescribed generally yet their biologically dynamic compounds are unidentified because of their efficiency and no consequence in clinical practice. Microorganism's resistance to the majority of antibiotics is quickly spreading. As a result there is a critical requirement for new antibiotics. Nearly all antibiotics have been obtained from microorganism

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