

RESEARCH ARTICLE

INDIAN KNOWLEDGE SYSTEM OF USING *ILICIIUM VERUM*.L WOUND HEALING IN DIABETIC FOOT ULCER – A TRADITIONAL APPROACHNirubama K^{1*}, Rekka R² Sharmila R³, Muneefa K I⁴, Sbari R⁵^{1*}Assistant Professor, Department of Biochemistry, Kongunadu Arts and Science College, Coimbatore., TamilNadu, India. Email address: nirubamak@kongunaducollege.ac.in²Assistant Professor, Department of Botany, Kongunadu Arts and Science College, Coimbatore, TamilNadu, India.^{3,4,5}Research Scholar, Department of Biochemistry, Kongunadu Arts and Science College, Coimbatore, TamilNadu, India.

ABSTRACT

Since its beginning, Indian Traditional Medicine, the cornerstone of the world's ancient medical practice, has been vital to human health and well-being. Traditional medicine in India refers to the use of medicines that are believed to have Indian origins or that were imported to India and assimilated into Indian culture. Ayurveda, Siddha, Unani, Yoga, Naturopathy, and Homoeopathy are just a few examples of the country's recognized traditional medicine. The need for natural products and plant-based medications is rising globally these days. Herbal preparations can be used for extended periods of time due to their non-toxic nature, which also makes them potentially more effective than conventional medications. This study investigated the phytochemical, antioxidant, and antimicrobial effects of *Illicium verum*.L. Phytochemical analysis, total antioxidant capacity, DPPH radical scavenging effect, Nitric oxide scavenging, Hydrogen peroxide radical scavenging were studied by established methods. Antibacterial, antifungal effects were screened by disk diffusion respectively Significant (P <0.05) IC50 values compared to respective standards were recorded in DPPH radical scavenging (54.141µg/ml), Nitric oxide scavenging (93.542 µg/ml), Hydrogen peroxide radical scavenging (93.542 µg/ml) methods. In antibacterial screening, the extract showed significant (P <0.05) zone of inhibitions compared to positive controls Chloramphenicol against Gram Positive *Enterococcus faecalis* and *Staphylococcus aureus* and Gram negative *Escherichia coli* and *Klebsiella pneumoniae*. In antifungal assay, the greater zone of inhibition was obtained for growth of *Candida albicans*.

Keywords: *Illicium verum*.L, Phytochemical, Bioactive compounds, Antioxidant, Antibacterial.

1. Introduction

Plants are an important part of the traditional medical system, used to cure a variety of infectious and non-infectious diseases all over the world. They are a valuable source of drugs due to their abundance of bioactive chemicals such as phenols, terpenoids, and alkaloids. In general, the usage of herbal remedies for treating various disease conditions is more widespread in rural areas where there is limited access to food and medical services. People typically consume plants in a variety of ways, including infusions, spices, and medicinal smoke.

Chronic inflammation, poor vascularization and tissue regeneration, decreased growth factor synthesis, high protease activity, and oxidative stress are all brought on by the hyperglycemic state at the wound site. Controlling bacterial infections is therefore crucial to the usual therapy of diabetic wounds, which also includes wound debridement, revascularization, and accelerating the healing process. The risk of progressive infection cannot

always be decreased by topical antimicrobial treatments (such as silver nitrate or povidone-iodine) or systemic antibiotic therapy (such as silver sulfadiazine, mafenide, mupirocin, or bacitracin), particularly if the bacteria resistant to antibiotics. In particular, reducing the duration of wound healing is essential for diabetics in order to minimize the risk of infection and reduce complications and expenses. Herbal products and the active ingredients in them have the potential to stop the growth of germs and to be very useful in treating resistant microbial strains. Also, some herbal products affect wound healing activities through anti-inflammatory and antioxidant activities, cell proliferation, and angiogenesis. The purpose of this study is to provide on the current knowledge acquired in herbal products (formulations and dressings) with diabetic wound healing activity. Moreover, herbal products and their active constituents used for microbial diabetic wound infections, and the various cellular and molecular mechanisms of their actions will also be described.

Antioxidant defense mechanisms protect all aerobic organisms from the damaging effects of free radicals. Antioxidants must be provided from outside sources if the antioxidant defense mechanism fails. Antioxidants are the substances that may protect the cells from the oxidative damage caused by free radicals. Natural goods may be good for human health and have significant antioxidant activity. Numerous plant species and their active components have been studied to find naturally occurring antioxidants with pharmacological qualities. Over the past few decades, there has been a growing interest in the study of the therapeutic qualities of many plants because of their strong pharmacological activities, ease of use, feasibility from an economic standpoint, and low toxicity. Although several studies on *Illicium verum*.L are currently ongoing, the extract has not yet been employed as an antioxidant compound for skin care. Thus, the current work focused on developing a formulation that can scavenge free radicals and protect against oxidative damage.

Star anise also known as Chinese SA, belongs to the Magnoliaceae family and is an aromatic plant. *Illicium verum*.L has also been researched for its antimicrobial, anti-inflammatory, anthelmintic, and gastro protective properties. In this work, we reported on the next steps, in which the ethanol extract of *Illicium verum*.L was analysed for phytochemical status, total antioxidant capacity, DPPH radical scavenging impact, Nitric oxide scavenging, and hydrogen peroxide radical scavenging effect. This study also reported on the antibacterial and antifungal properties of the leaf extract using reference standards in each case. Thus, the result shows that *Illicium verum*.L has good biological activity, and further it can be carried out in pharmaceutical industries. From these results, it could be concluded that the prominent bioactive compounds in tested this spice have the potential to treat a wide range of serious diseases. These findings suggest that *Illicium verum*.L holds promise as a source of natural emollient.

Materials and Methods

Sample Collection and Preparation

The plant *Illicium verum*.L was collected during the month of January 2024 from Coimbatore, Tamil Nadu, India. The plant was identified and authenticated by Department of Botany Kongunadu Arts and Science College Coimbatore. The collected *Illicium verum*.L was washed thoroughly with distilled water and dried for 7 days in room temperature. The dried sample was ground to coarse powder with a mechanical grinder and powered sample were kept in clean closed container. The powered sample was subjected to ethanol extraction. 25g of powered sample was mixed with 250ml of ethanol in Soxhlet and the extract was used for phytochemical.

Chemicals

Totally Four bacterial strains and one fungal strain were used throughout investigation. All the bacterial and fungal cultures were obtained from

Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, and Chandigarh, India. The bacteria used were *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*. The fungal strains used were *Candida albicans*.

Qualitative analysis

Qualitative phytochemical analysis of each of *Illicium verum*.L was carried out by using Ethanol solvent to identify the major natural chemical groups such as carbohydrates, tannins, saponins, flavonoids, alkaloids, glycosides, terpenoids, phenols, steroids and as per the procedures.

Preparation of Cream

Take the grated beeswax (3g) in a beaker and double boil it. After the beeswax is melted add 5ml white liquid paraffin and stir the solution for 5 minutes. Now add 5ml of *Illicium verum*.L extract. Stir it well and add 0.5 g of zinc oxide to the solution. Cool the beaker for few minutes. After cooling keep the cream in a air tight container.

In-Vitro Characterization of Cream Formulation

Cream pH was recorded with a digital pH meter (Mettler & Toledo et al 2014) by inserting probe the cream formulation and allowing it to equilibrate for 1 minute. Viscosity were conducted using a Model RVTDVII Brook field viscometer (Stoughton, MA) AC-50 spindle was employed with a rotation rate of 220 rpm. The gap value was set to 03 Temperature was set at $25^{\circ}\text{C} \pm 2$ and these experiments were conducted in triplicate to statistically significant data.

The spreadability of the cream was determined by the wooden block and glass slide previously detailed somewhere else. Essentially, a 5ml. volume (100 mg) of cream was added to a dedicated pan and the time taken for a movable upper slide to separate completely the fived slides was noted

All the formulated cream were subjected to a 6 month-long protocol of accelerated stability conducted at a temperature of $40 \pm 2^{\circ}\text{C}$, 75% relative humidity. The accelerated stability was performed in accordance to the ICH guidelines. At 12h, 1day, 7days, 1month, 3 and 6 months, each formulation was examined for changes in appearance, pH, viscosity drug content.

The formulations were evaluated with different evaluation parameters like colour, odour state, consistency, pH, spreadability, washability, non-irritancy test phase separation test, After feel, In vitro permeation studies, Patch test. The objective of this review is to compile the information of herbal formulations of cream and its evaluation Herbal cream formulations studied by many researchers and this information can be researchers for novel herbal cosmetic formulations with new herbs.

In Vitro Antioxidant Activity

DPPH Radical Scavenging Assay

The antioxidant potential of the plant extract was determined by DPPH method. The antioxidant activity of the extract was assessed on the basis of the radical scavenging effect of the stable DPPH free radical. Then a volume of 1.9ml of DPPH solution was added into a test tube and 100ul of the plant extract was added to it (At). The mixture was kept in dark for 30 minutes at room temperature. A solution containing 1.9ml methanol and 100ul of plant extract was taken as the test blank. A volume of 2.0ml of DPPH solution was taken as the control (Ac) and a volume of 2.0ml of methanol was used as the control blank. All of the sample were incubated in dark for 30minutes at room temperature and the absorbance values of these samples were measured at 517nm. Then percentage DPPH radical scavenging concentration. All experiments were carried out in triplicate. Percentage inhibition (%) = $\frac{[(Ac)-(AT)]}{Ae} \times 100$ Ac Absorbance of the control sample at 540nm, AT Absorbance of the test sample at 540nm.

NO Radical Scavenging Assay

The procedure is based on the method, where sodium nitroprusside in aqueous solution at physiological pH, spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions. Take 100 µl of plant extract and standards (BHT and rutin) in triplicates. Add 3 mL of sodium nitroprusside (10 mM) to the extracts in test tubes. Incubate all the test tubes at room temperature for 150 min. Add 3mL of Griessreagent (1% sulphaniilamide, 2% H₃PO₄ and 0.1% N-(1-naphthyl) ethylene diaminedihydrochloride) to all the test tubes. The same reaction mixture without the sample is the negative control. A test tube with phosphate- buffered saline alone will act as blank. Read the absorbance of the chromophore formed at 546 nm against the blank.

Hydrogen Peroxide Radical Scavenging Assay

Hydrogen peroxide radical scavenging activity of the test sample was estimated by the method of Ruch et al. (2018). A solution of hydrogen peroxide was prepared in phosphate buffer (pH 7.4) 200.0 µl of sample containing different concentration was mixed with 0.6 ml of H₂O₂ solution. Absorbance of H₂O₂ was determined 10 minutes later against a blank solution containing phosphate buffer without H₂O₂. A test tube containing 200µl of phosphate buffer and processed as described above served as the control tube. Different concentration of ascorbic acid was used as reference compound.

In Vitro Anti-Microbial Activity

The well diffusion method was used to screen the antimicrobial activity. *In vitro* anti microbial activity was screened by using Muller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petri plates. The plates could solidify for 5 minutes and 0.1% inoculums suspension was swabbed uniformly, and the inoculums could dry for 5 minutes. Wells were cut and 20 µl of the different concentration of test drug were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). Chloramphenicol disc was used as a positive control.

Anti - Fungal Activity

Antifungal activity was measured using methods of well diffusion plates on agar. To test the antifungal activity, the fractions of different concentration of plant extract were dissolved in 70% ethanol. 20 mL of Sabouraud Dextrose Agar was poured into each 15 cm Petri dish. *C. albicans* were grown in sabouraud dextrose broth at 27 ° C for 48 h. Growth was adjusted to OD (600 nm) of 0.1 by dilution with sabouraud dextrose broth. Then, Wells were cut and 20 µl of the different concentration of test drug were placed on agar to load 10 and 15 µL of each spice sample (1 mg/mL). 100 units of Fluconazole, obtained from a local pharmacy, were used as a positive control. Inhibition zones were determined after incubation at 27 ° C for 48 hrs.

Results and Discussion

Photochemical are the derivatives present in the plants are promising options to improve treatment efficiency in Diabetic foot ulcer patients and decrease adverse reactions. A number of these photochemical are naturally occurring biologically active compounds with significant ant diabetic potential. (Amit choudari *et al.*, 2020)

Therapeutic efficacy of any medicinal plant depends upon the quality and quantity of the active phytoconstituents, which vary with latitude, altitude, climate and season. Different parts of these plants may possess different level of pharmacological activity. Additive or synergistic effects of bioactive phytoconstituents may be responsible for the concerned pharmacological function rather than the purified one. (Singh Sukhdev *et al.*, 2016) Scientific evidence indicate that photochemical have significant anti diabetic potential. (Newman and Cragg *et al.*, 2016).

Alkaloids are important chemical compounds that serve as a rich reservoir for drug discovery. Several alkaloids isolated from natural herbs exists anti proliferation and anti metastasis effect on various types of diabetes both in-vitro and in-vivo.

Terpenoids found in a variety of plants. Fruits, vegetables, spices, plant derived beverages such as green tea, wine and cocoa-based products are the main dietary sources of flavonoids. Terpenoids have been shown to possess a wide variety of anti-diabetic effects. The value of medicinal plants lies in some chemical substances that produce a definite

physiological action on the human body and the most important phytochemicals are alkaloids, flavonoids, tannins and phenolic compounds. The Medicinal plants have potent phytochemical components which are important source of antibiotic compounds and are responsible for the therapeutic properties (Florence et al., 2020).

Table 1: Phytochemical Analysis of ethanolic extract of *IlliciumVerum.L*

Phytochemical	Tests	<i>IlliciumVerum.L</i> (Ethanol Extract)
Carbohydrate	Molisch'sTest	+
Protein	NinhydrinTest	-
Alkaloids	Hager'sTest	+
Saponins	Lieberman-Burchard	+
Steroids	ForthTest	-
Tanin	FerricChlorideTest	+
Flavanoids	AlkalineReagent Test	-
Glycoside	CardiacGlycosides Test	+
Phenols	LeadAcetate Test	+
Terpenoids	Salkowski Test	+

Preparation of Cream

To formulate and evaluate herbal body cream using beeswax, white liquid paraffin, Zinc Oxide, *Illicium verum.L* to give multipurpose effect. Creams maintain skin's hydration levels by locking in the moisture, keeping the skin healthy, soft, and supple and also heals the wounds. Unlike a lotion, the

creams are less greasy and have more water content.

Beeswax exfoliates, conditions, soothes, and calms the skin, white liquid paraffin eradicates skin bacteria and remove dead skin cells and act as a emollient, *Illicium verum.L* extract fight skin infections, promote wound healing, zinc oxide acts as a good moisturizer.



Fig 1: Preparation of *Illicium verum.L*

***In - Vitro* Studies of *Illicium Verum*.L Cream**

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infections, promote wound healing, zinc oxide acts as a good moisturizer.

The colour and odour of the cream was found to be attractive for use. The pH 6.9 make the cream suitable for all skin type as it in basic range. The absorbing capacity of the cream by the skin is high due to its semi solid state. The viscosity value of the product is measured as 6.4g.cm/cm, which exhibits a good spreadability score. The other parameters like washability, non irritancy and phase separation where found to be negative. The cream provided a soothing feel after its application, which is indicated as emollient.

Table 2: *In vitro* study and evaluation of *Illicium verum* cream

S. NO	PARAMETERS	RESULT
1	Color	White brown
2	Odor	Anise like odor
3	State	Semi-solid
4	Consistency	Smooth
5	PH	6.9
6	Spreadability Test	6.4g.cm/cm
7	Washability Test	Not easily washable
8	Non-Irritancy Test	Non irritant
9	Phase Separation Test	No phase separation
10	After Feel	Emollient

Antioxidant and Free Radical Scavenging analysis of *Illicium verum*.L Cream By Radical Scavenging Assay

Several concentrations ranging from 10-250 µg/ml *Illicium verum*.L were tested for their antioxidant activity in different in vitro models. The percentage of inhibition was observed and found that free radicals were scavenged by the test compounds in a concentration dependent manner up to the given concentration in all the models.

Natural antioxidants are widely utilized in the food and medicine industries as they counteract the cellular free radicals. Antioxidant capacity is important marker for assessing medicinal bioactive components. A variety of methods are used to study antioxidant potential of medicinal plants. Owing to

the different chemical nature and complexity of antioxidant compounds present in extract of medicinal plants, there is variation in their mode of actions, so more than one assay are advised for evaluation of antioxidant activity. In this study three radical scavenging (DPPH, Hydrogen Peroxide, Nitric oxide) and reducing power assays were performed to determine the antioxidant potential. DPPH radical Scavenging Assay

The important property of an antioxidant is its ability to scavenge free radicals. DPPH radical scavenging is one of the most commonly used method for assessment of antimicrobial activity of medicinal plants. The DPPH method is simple and time saving method. DPPH contains an odd electron which gives absorption maximum at 517 nm and is

purple in colour. When freeradical scavenging antioxidants (phenolics) donates the hydrogen to freeradical, it becomes paired with hydrogen and formed reduced form of DPPH (Gulcin et al. 2019, Bahramikia et al., 2018). After reduction, the colour of DPPH is changed from purple to yellow This discoloration is stoichiometric with respect to radical scavenging activity. The DPPH radical scavenging activity was detected and compared with Ascorbic Acid. The activity of DPPH radical scavenging of *Illicium verum*.L and ascorbic acid was presented. The percentage of inhibition in DPPH in different concentration like 10, 50, 100, 150 , 200

and 250 µg/ml were observed in 32.32, 42.86, 49.29, 52.28 and 54.93 respectively whereas the percentage inhibition of ascorbic acid concentration like 50, 100, 150, 200 and 250 µg/ml were found to be 32.6 , 43.53 , 59.96 , 69.23 , 79.56 respectively. The IC₅₀ values for DPPH scavenging activity for *Illicium verum*.L and ascorbic acid were 54.141 µg/ml and 93.542 µg/ml. The higher inhibition activity was recorded in *Illicium verum*.Lin dose dependent manner Values are the average of triplicate experiments and represented as mean standard deviation.

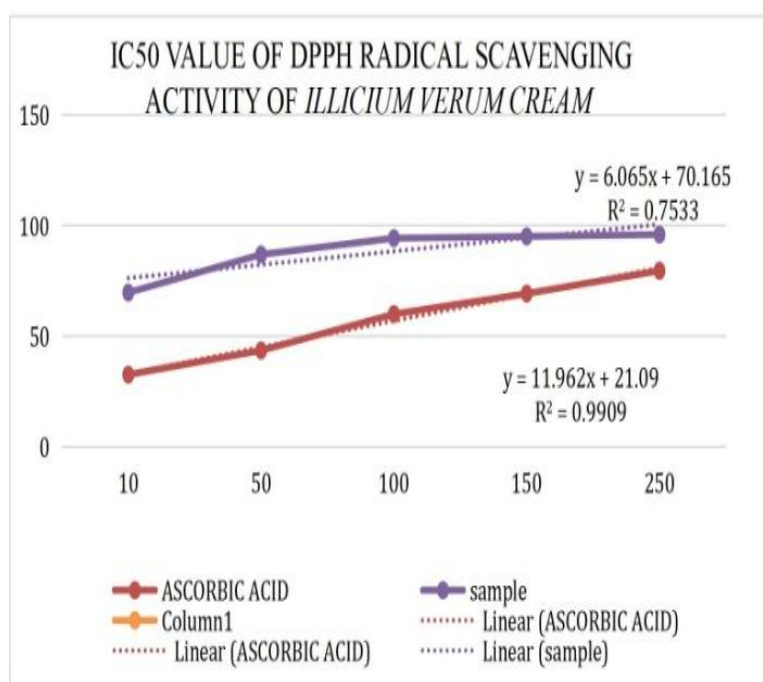


Fig 2: Graphical representation of DPPH Radical Scavenging activity of *Illicium verum* cream

H₂O₂ Scavenging Assay

The H₂O₂ Scavenging activity was detected and compared with Ascorbic acid the activity of H₂O₂ scavenging of *Illicium verum*.L and Ascorbic acid was presented in Figure. The percentage of inhibition in H₂O₂ in different concentration like 10, 50, 100, 150 and 250 µg/ml were observed in 32.6, 43.53, 59.96, 69.23 and 79.56 respectively whereas the percentage inhibition of Ascorbic acid in concentration like 10, 50, 100, 150 and 250 µg/ml were found to be 15.21 , 31.50 , 33.05 , 54.53 , and 55.20 respectively. The IC₅₀ values for H₂O₂ scavenging activity for *Illicium verum*.L and ascorbic acid was 93.542 µg/ml and 65.350 µg/ml. The higher inhibition activity was recorded in *Illicium*

verum.Lin dose dependent manner. Values are average of triplicate experiments and represented as mean standard deviation (Lawenda et al 2018).

Free radical generation is a normal physiological process with a variety of effects. But increased production of these free radicals will render the lipids susceptible to lipid peroxidation. A common reliable marker of lipid peroxidation is malondialdehyde (MDA) which is measured Thiobarbiturate assay Evolution has also provided the cells with a number of counter acting antioxidant defense. These antioxidant defense mechanisms can be categorized in to two types- free radical scavenging and chain breaking antioxidants (Rosey Lekhru et al., 2017).

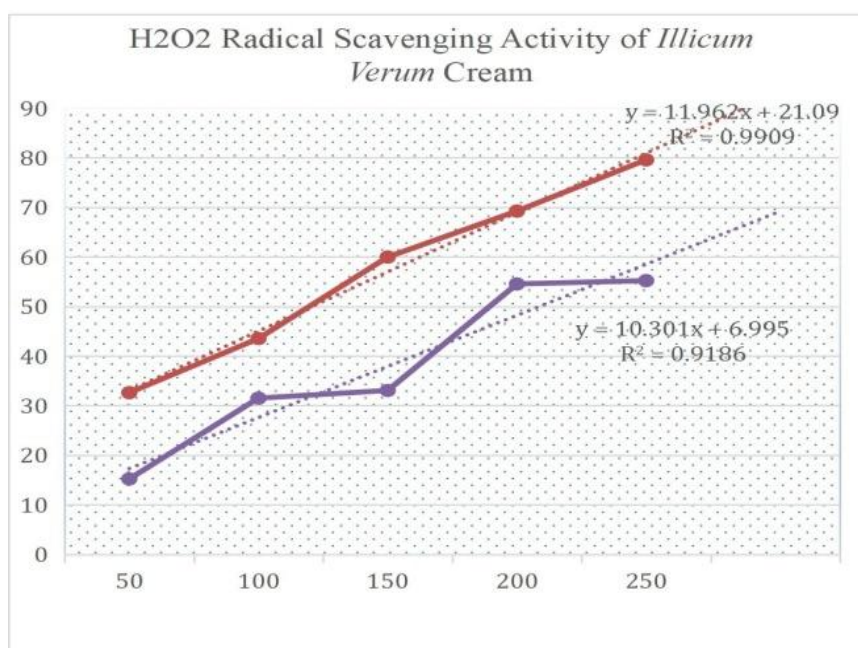


Fig 3: Graphical representation of H2O2 Scavenging Activity *Illiciumverum* cream

NO Radical Scavenging Assay

Nitric oxide is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signalling, inhibition of platelet aggregation and regulation of cell mediated toxicity. This extract inhibits nitric oxide in a dose dependent manner (Rana et al.,2010) . The nitric oxide radical scavenging activity of 80% ethanol extract of

Illicium verum.L. The concentration was taken with 50, 100, 150, 200 and 250 µg/ml, produce a dose dependent scavenging of nitric oxide radicals. The effect was compared with standard ascorbic acid, the maximum scavenging effects of nitric oxide radical was obtained at 77.73 and 79.56% of inhibition in 250 µg/ml and IC50 values were found to be 93.542 and 86.120 µg/ml

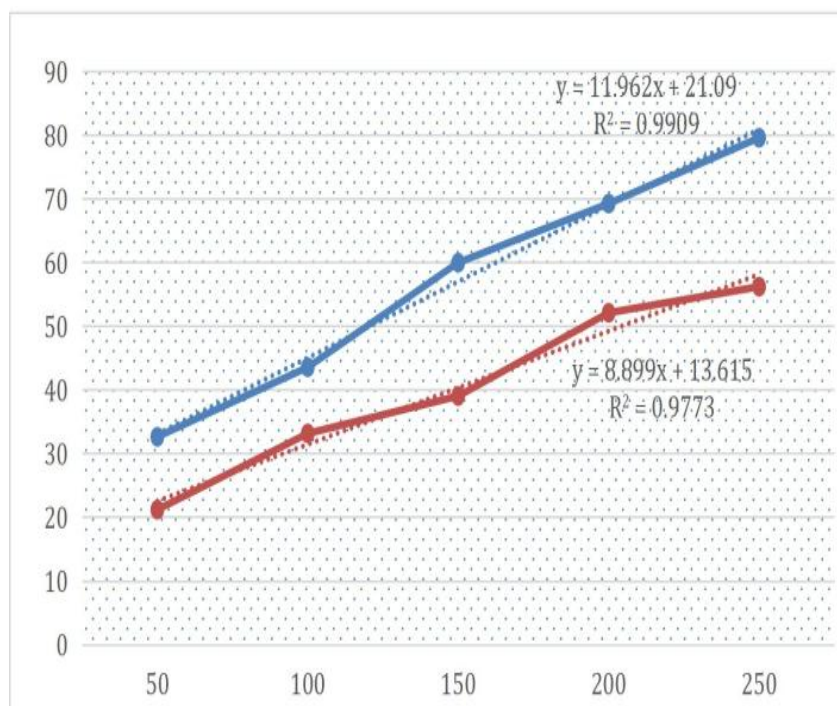


Fig 4:Graphical representation of Nitric Oxide radical Scavenging of *Illicium verum* cream

Anti -Microbial activity of *Illicium Verum*.L Cream

Results of antifungal activity showed almost similar trends as antibacterial activity. The results of this study revealed that diameter of the zone of inhibition for fungal strains was less than the diameter measured for bacterial strains. *Illicium verum*.L showed greater inhibitory effect on

bacterial strains as compared to fungal strains. This distinction is due to difference in cell wall structure and protein synthesis of fungal and bacterial strains. These findings are in agreement with the observations of many other researchers (Papadopoulou *et al.*, 2005). The greater zone of inhibition was obtained for *Staphylococcus aureus* with 22mm.

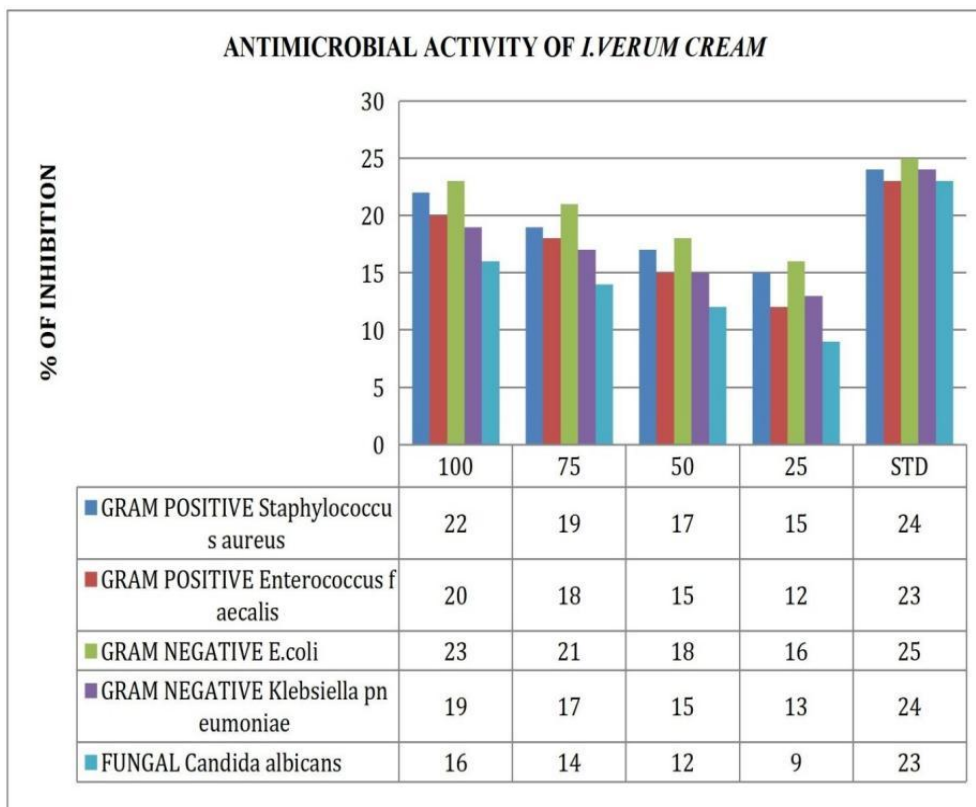


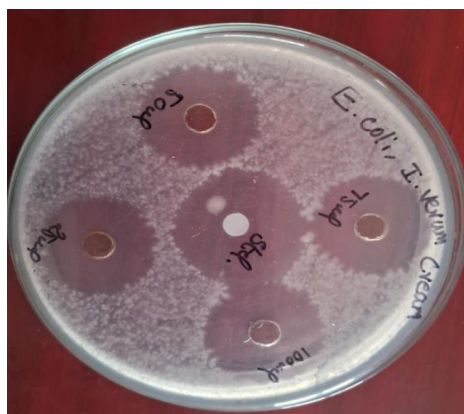
Fig 5: Graphical representation of antimicrobial activity of *I.Verum* Cream



Staphylococcus aureus



Enterococcus faecalis



E. coli



Klebsiella pneumoniae

Candida albicans

Fig 6: Diagrammatic representation of antimicrobial activity of *illiciumverum* cream.

Conclusion

Herbal products and their dynamic ingredients through different mechanisms of action, including antimicrobial, anti-inflammatory, and antioxidant activities. The present study was formulated cream to understand the antimicrobial, antioxidant and phytochemical properties of the cream *Illicium verum*.L which is identified alkaloid, saponin, flavanoids and glycoside type compounds, Based on the results obtained in the present study, it might be *Illicium verum*.L cream exhibits high anti - fungal and kills by preventing the growth of microorganism and exhibits high anti-oxidant activity and scavenging of free radicals. It is may be considered as an important support during conventional therapy or even as a substitute for synthetic drugs used for diabetic wounds treatment. Better quality control techniques for identification, screening and quantification herbal components along with well-designed pre-clinical and clinical studies will open new research gateways in wound management.

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