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Formulation and Evaluation of Herbal Transdermal Patches Loaded with Extract of *Asparagus racemosus* for Anti-Bacterial Activity

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ABSTRACT:

Introduction: Transdermal drug delivery systems have emerged as a promising alternative to conventional routes due to their ability to offer controlled and sustained drug release, enhanced bioavailability. Asparagus racemosus (Shatavari), a medicinal plant known for its antimicrobial, anti-inflammatory, and adaptogenic properties, has been incorporated into transdermal patches in this study to evaluate its efficacy in antibacterial applications. **Method and Methodology:** Methanolic extracts of Asparagus racemosus roots were prepared using Soxhlet extraction. Four different formulations (F1–F4) of herbal transdermal patches were developed using varying concentrations of polymers such as PEG 4000, PVA, PVP, EC, and HPMC. The patches were subjected to detailed physicochemical evaluations including thickness, folding endurance, surface pH, drug content, and moisture content. MIC values were also determined to assess the minimum effective concentration of the extract. **Result:** All four formulations exhibited acceptable physicochemical properties. Notably, formulation F4 demonstrated superior performance with the highest drug content (88% ± 1.24), consistent thickness (0.17 ± 0.77 mm), excellent flexibility, and high folding endurance (31 ± 1.24), making it the most stable and robust patch. The antibacterial assay showed the highest zone of inhibition (10 mm) against Staphylococcus aureus, indicating effective antimicrobial potential. The enhanced performance of F4 is attributed to the inclusion of HPMC, which contributes to better film-forming capacity, elasticity, and drug retention. MIC analysis confirmed the extract's efficacy at concentrations as low as 15.62 mg/mL. **Conclusion:** The study successfully developed and evaluated Asparagus racemosus-loaded herbal transdermal patches. Among all, formulation F4 proved to be the most effective in terms of drug content, mechanical strength, and antimicrobial activity. The findings support the potential of F4 as a promising candidate for further development as a herbal-based transdermal therapeutic system for topical antibacterial applications.

Keywords: Transdermal patch, Comparison, *Asparagus racemosus*

INTRODUCTION:

Since ancient times, plants have served as significant sources of medicinal remedies. The use of plants to heal a variety of human ailments is mentioned in Ayurveda and other Indian literature. Drugs used in Indian medicine and other traditional medical systems around the world are mostly derived from plants. The Rig Veda, Charaka Samhita, and Sushruta Samhita provide the first descriptions of the therapeutic qualities of medicinal plants. They also include detailed descriptions of a variety of medicinal herbs. In India, information about medicinal plants offers a wealth of information regarding traditional uses of medicinally significant natural items as well as folklore. Indian traditional medicine is founded on a number of systems, such as homoeopathy, Siddha, and Ayurveda (Shahana Jabi, 2024).

Plants have been used as a warning source of medicine since prehistoric times. Plants are used to heal a variety of human ailments, according to Ayurveda and other Indian literature. In the Indian medical system and other traditional medical systems around the world, plants have been the primary source of pharmaceuticals. The first account of medicinal plants' therapeutic qualities can be found in the Rig-Charaka Samhita, Sushrusha Samhita, and the Veda, which provide detailed descriptions of a variety of therapeutic herbs. Information about Indian medicinal plants has been arranged in a methodical manner (3–7). Traditional medicine has a long history in India. A wealth of knowledge about the traditional uses and mythology around medicinally significant natural items can be found in India's materia medica. Indian traditional remedies derived from Siddha, Unani, and Ayurvedic systems.

Consequently, the purpose of this work is to provide a summary of traditional phytochemical, pharmacognocal, and traditional studies conducted on the roots of the Asparagus recemosus Willd. plant (Md. Parwez Ahmad, 2017).

Transdermal patches are medicated adhesive patches that are put to the skin to gradually transfer a prescribed dosage of medication into the bloodstream through the skin. Compared to oral or injectable routes, this drug delivery system provides a regulated release of medication, increasing convenience and compliance. Transdermal patches are frequently used to treat cardiovascular disorders, motion sickness, hormone therapy, nicotine replacement therapy, and pain management (such as fentanyl). They improve therapeutic efficacy by avoiding the liver's first-pass metabolism, lowering gastrointestinal side effects, and supplying consistent drug levels.

A backing layer, medication reservoir, adhesive layer, and protective liner are usually included in these patches. Iontophoresis and microneedles are two cutting-edge technologies that are improving drug absorption even more. Although they have several benefits, their usage is limited to certain drugs because of issues such as skin irritation and low drug permeability. All things considered, transdermal patches are a practical, non-invasive, and efficient way to administer medication. Transdermal patches are medicated adhesive patches that are put to the skin to gradually introduce medications into the bloodstream. It improves patient convenience and adherence by delivering a steady and regulated delivery of medication. These patches are frequently used to treat cardiovascular disorders, hormone therapy, nicotine replacement, and discomfort. They have benefits like consistent drug levels, fewer adverse effects, and increased drug efficacy due to avoiding the digestive system (Jain, 2005).

Pharmacognosy of Asparagus racemosus

In Ayurveda, the herb Asparagus racemosus, also called Shatavari, is used as a medicine. It is prized for its immunomodulatory, galactagogue, and adaptogenic qualities and is a member of the Asparagaceae family. Mucilage, alkaloids, flavonoids, and steroidal saponins are among the bioactive substances found in the plant. Because of their anti-inflammatory, antioxidant, and anti-ulcer qualities, the roots are mostly utilised medicinally. It has long been used to improve immunity, aid with digestion, and promote female reproductive health. Its roots are tapered, cylindrical, and tuberous at the macroscopic level. Mucilage, lignified vessels, and starch grains are visible under a microscope. It is important in herbal therapy because of its pharmacological properties.

Why Asparagus Racemosus in Trans Dermal Patches

1. **Presence of Bioactive Compounds:**

Asparagus racemosus is rich in saponins, alkaloids, and flavonoids, contributing to its medicinal properties (Chanchal Malik, 2023).

2. **Enhanced Drug Permeation:**

Studies have shown that Asparagus racemosus extract can enhance the permeation of drugs like carvedilol across the skin, indicating its potential as a natural penetration enhancer (Bharti Sapra, 2009).

3. **Sustained and Controlled Release:**

Transdermal patches incorporating Asparagus racemosus extract have demonstrated improved performance in controlling hypertension in rats, suggesting their efficacy in providing sustained and controlled drug release.

4. **Avoidance of First-Pass Metabolism:**

Transdermal delivery bypasses the gastrointestinal tract and first-pass metabolism, enhancing the bioavailability of bioactive compounds in Asparagus racemosus (Bharti Sapra, 2009).

5. **Potential for Wound Healing & Anti-inflammatory Effects:**

The antifungal and anti-inflammatory properties of Asparagus racemosus make it a suitable candidate for topical applications targeting skin conditions such as dandruff and seborrheic dermatitis.

6. **Biocompatibility & Safety:**

Asparagus racemosus has been used traditionally and is considered possibly safe when taken orally at appropriate doses.

7. **Stability in Formulation:**

The incorporation of Asparagus racemosus extract into transdermal systems has been studied, indicating its stability and effectiveness in enhancing drug permeation (C Onlom, 2014) (Sapra, 2009).

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Figure 1: Roots of ***Asparagus racemosus***

METHOD AND METHODOLOGY:

A. Collection and Authentication: Root of *Asparagus racemosus* (white satavri) were collected from the local area of Sidhari, Azamgarh, Uttar Pradesh, washed with sterile water and dried in shades. Then the samples were powered in mechanical grinder. The plants were examined by Prof. Nawal Kishore Dubey (FNASc, FNAAS, Centre of Advanced Study in Botany, Institute of Science, Banaras Hindu University, Varanasi-221005.

B. Extraction (Soxhlet Apparatus): The powdered material of *Asparagus racemosus* was extracted by methanol. A known amount of powdered material (80gm) of *Asparagus racemosus* was taken in two separate assemblies simultaneously. The powdered material was subjected to Soxhlet extraction and exhaustively extracted with respective solvents for about 48 hours. The extracts were filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator and dried in the desiccator. The solvent was removed under pressure to obtain a total extract (Haque, 2020) (Jeniffer Torres-Vega, 2020).

- **Materials and Ingredients-** (*Asparagus racemosus)*

➢ *Asparagus racemosus* root powder (around 80 grams)

➢ Methanol

➢ Mortar and pestle or grinder

➢ Glass container with a lid

➢ Filter paper or a fine mesh strainer

➢ Funnel

➢ Glass beaker

➢ Evaporating dish or similar container

➢ Water bath or gentle heat source

- **Procedure:**

1. Grind the Asparagus Racemosus root powder to increase its surface area. You can use a mortar and pestle or a grinder for this.
2. Place the ground Asparagus Racemosus root powder in a glass container with a lid.
3. Add enough methanol to completely cover the licorice root powder. Ensure there is enough ethanol to facilitate extraction.
4. Seal the container with the lid and shake it gently to mix the solvent and plant material.
5. Store the container in a cool, dark place for several days to allow for extraction. Agitate or shake the mixture occasionally during this period.

6. After the extraction period, filter the mixture to separate the liquid extract from the solid plant material. You can use filter paper or a fine mesh strainer for this purpose.

7. Collect the liquid extract in a glass beaker.

8. To remove the methanol, you can use a water bath or gentle heat source to evaporate it. Be cautious not to overheat the extract, as glycyrrhizin can be sensitive to high temperatures. Alternatively, you can use a vacuum pump or rotary evaporator for ethanol removal if available.

9. Once the methanol has been removed, you should be left with glycyrrhizin extract. Transfer this extract to an evaporating dish or similar container.

10. Allow the extract to air dry in a controlled environment to remove any residual ethanol.

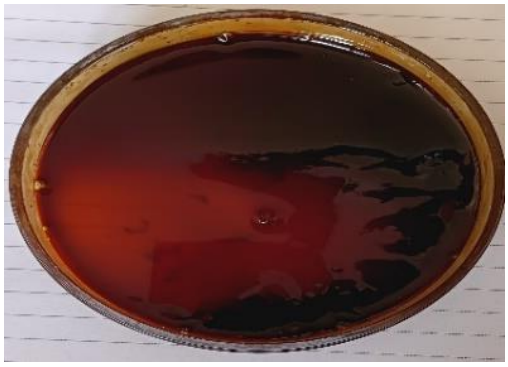

Figure 2: Extract of *Asparagus Racemosus*

C. Preliminary Phytochemical Analysis:

1. **Phytochemical testing for Qualitative:** The table makes it clear that the highest concentration of chemical elements, such as carbohydrates, alkaloids, flavonoids, sterols, glycosides, amino acids, and saponins, was found in the methanol extract. Since steroidal chemicals are in charge of a number of biological processes in the human body, their presence is significant in medicinal applications. This could be the cause of its usage in milk secretion facilitation and in lowering mammary gland distension. This plant may have antioxidant qualities because it contains flavonoids, which are thought to be effective free-radical scavengers. Glycosides are linked to decreasing blood pressure, while saponins are linked to antimicrobial action (Y.C Tripathi, 2015).

2. **Fluorescence Analysis (UV Lamp):** Plant material (such root) is dried, ground up, and then exposed to a variety of solvents or reagents, including ethanol, chloroform, hydrochloric acid (HCI), sulphuric acid (H2SO4), methanol, petroleum ether picric acid sodium hydroxide Iodine, ferric chloride (fecl3) and Distilled water, in order to perform the fluorescence test. In order to identify and characterize the chemical components present in plant material, the treated samples are analyzed under ultraviolet (UV) light to detect fluorescence (amruta jadhav, 2015).

3. **Powder Microscopy (Compound Microscope):** Examining powdered samples under a microscope to ascertain their physical and chemical properties is known as powder microscopy. This method is frequently used in disciplines including materials research, forensic science, and pharmacognosy. Particle size and shape, crystallinity and crystal structure, homogeneity, purity and impurities, chemical content, surface morphology and texture, and thermal properties can all be analyzed with its help (WHO, 1998).

D. Physio-Chemical Analysis:

- **Ash Value Determination (Mount cento furnace**): Weighing two to three grammes of the sample precisely is the first step in determining the total ash value of plant leaf dry powder. After the sample has been weighed, place it in a crucible and gradually burn it at 500–600°C in a muffle furnace until it turns white, signifying that all of the carbon has been eliminated. To prevent moisture absorption, cool the crucible in a desiccator after cremation. Weigh the crucible with the ash once it has cooled. The following calculation is then used to determine the total ash value.

%ASH = [(ashed wt.) – (crucible wt.)] × 100 \ [(crucible and sample wt.) – (crucible wt.)] **Eq. 6**

- **Swelling index determination:**

A 100 ml graduated cylinder containing 1 gram of mucilage powder was used to calculate the swelling index using the BP method. After that, 25 cc of water was added, and for an hour, the mixture was shaken every ten minutes. After then, the mixture was left to settle for a full day. By averaging the three measurements and dividing the result by the total amount of mucilage, the swelling index was determined (Namade C. T. Baste N, 2014) (Wilson).

Swelling index = V2 – V1\ W1 Eq. 1

Were, V2: Final volume of the mixture

V1: Initial volume of the water

W1: Weight of the powder

- **Angle of Repose:** The flow characteristics of the powder are gauged by the angle of repose. It is the greatest angle formed between the horizontal plane and the surface of the powder heap. The angle of repose was calculated using the formula. A funnel with a set height was used to apply the finely ground mucilage to graph paper. An equation was used to determine the angle of repose in accordance with the USP after the height and base of the created powder heap were measured (Sarvesh Kumar, 2020).

$$\tan\theta = h/r$$

$$\theta = \tan^{-1}(h/r) \qquad \textbf{Eq. 1}$$

Where, θ represents the angle of repose,

H is height in cm

R is radius/base in cm.

- **Bulk density (BD):** The ratio of a powder's bulk volume to its entire mass is known as its bulk density (BD). Pour 50 g of precisely weighed powdered mucilage into a 100 mL graduated barrel. The initial apparent volume (Vo) of mucilage in the combination was meticulously levelled. You can use the loose bulk density formula, and the result will be given in g/ml (Mohammed Shaibu Auwal, 2014).

$$\rho b = M/Vb \qquad \textbf{Eq. 2}$$

Where ρb=bulk dernsity, M=bulk weight of blend, Vb= bulk volume of the blend.

- **Tap density (TD)**: The mass of the powder divided by the volume tapped is known as the tap density (TD). Forty grams of the powder mixture have been put in a 100 mL container cylinder for measurement; weigh them properly. The sample-containing cylinder was manually tapped three times (1250, 750, and 500) before the final tapped volume (Vf) was measured. You can use the tapering bulk density approach, and the result will be given as g/ml.

$$\rho t = M/Vt \qquad \textbf{Eq. 3}$$

Where, ρt=Tapped density, M=weight of blend, Vb= tapped volume of the blend.

- **Compressibility Index (Carr's Index):** Carr's Index, also known as the Compressibility Index The ratio of bulk density to tapped density and the difference between the two is known as the compressibility index. It indicates and quantifies the proportion of powder flowability (Sakar B) (S.B., 52nd edition).

$$\text{Carr's Index (\%)} = (Dt\text{-}Db/Dt) * 100 \qquad \textbf{Eq. 4}$$

Where Dt = Tapped density of the powder, Db=bulk density of the powder.

- **Hausner ratio**: An indicator of a powder or granular material's flowability is the Hausner ratio. It's an indirect way to measure how easy it is for powder to flow. An indicator of a powder's or granular material's flow quality is the Hausner ratio. It is an indirect metric used to assess how easy it is for powder to flow (Sinha, 2018).

$$\text{Hausner ratio} = \rho b/\rho t \qquad \text{Eq. 5}$$

Where, ρb=Tapped density of the powder, ρt =Bulk density of the powder.

FORMULATION OF TRANSDERMAL PATCHES

Ingredients Use:

I. Polymer matrix or matrices:

The matrix of polymers the drug's release from the device is regulated by the polymer. For a polymer to be utilized in a transdermal system, it must meet the following requirements:

(i) The polymer's molecular weight, glass transition temperature, and chemical functionality should all be such that the particular medication diffuses and is released through it in an appropriate manner.

(ii) The polymer should be affordable, easily produced into the required product, stable, and non-reactive with the medicine.

(iii) The polymer and the byproducts of its breakdown must not be harmful or antagonistic to the host.

(iv) Adding a lot of active ingredients to the polymer shouldn't cause its mechanical qualities to deteriorate too much.

The following polymers could be helpful for transdermal devices:

- **Natural polymers**

Cellulose derivatives, Zein, Gelatin, Shellac, Waxes, Proteins, Gums and their derivatives, Natural rubber, Starch etc.

- **Synthetic elastomers**

Polybutadiene, Hydrin rubber, Polysiloxane, Silicone rubber, Nitrile, Acrylonitrile, Butyl rubber, Styrene-butadiene rubber, Neoprene etc.

- **Synthetic polymers**

Polyvinyl alcohol, Polyvinyl chloride, Polyethylene, Polypropylene, Polyacrylate, Polyamide, Polyurea, Polyvinylpyrrolidone, Polymethylmethacrylate, Epoxy etc. (Jain, 2005).

II.Plasticizers

Additionally, plasticizers have been utilized in a variety of formulations at levels between 5 and 20% (w/w, dry basis). Examples include dry basis, phosphate, phthalate esters, fatty acid esters, glycerol or sorbitol at 15% w/w, and glycol derivatives like PEG 200 and PEG 400 (Jyothsna Savula, 2017).

III.Solvents

These substances may improve penetration by fluidizing lipids or by enlarging the polar route. Examples include water alcohols, such as methanol and ethanol; alkyl methyl sulfoxides, such as dimethyl sulfoxide, dimethyl acetamide, dimethyl formamide, and alkyl homologs of methyl sulfoxide; pyrrolidones, such as 2-pyrrolidone, N-methyl, and 2-pyrrolidone; laurocapram (Azone); and various solvents, such as propylene glycol, glycerol, silicone fluids, and isopropyl palmitate (Jain, 2005).

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Figure 3: Blank transdermal patches

Table 1: Ingredient of Patches

S. No	Formulation	F1	F2	F3	F4
1	Methanolic extract (gm)	0.25	0.25	0.25	0.25
2	Methanol (ml)	3	3	3	3
3	PEG 4000 (%)	40	40	40	40
4	PVA (%)	5	5	5	5
5	EC (%)	1	1	1	1
6	PVP (%)	2	5	10	--
7	HPMC (%)	--	--	--	2

EVALUATION OF TRANS DERMAL PACTH

1. Organoleptic Characteristics

A visual inspection of the created patch's look, color, clarity, flexibility, and smoothness was used to assess its physical qualities.

A. Thickness of Patch

The homogeneity of patch thickness was measured at six distinct locations using a vernier caliper Next, it was calculated how thick each of the six locations was on average.

B. Determination of Surface Ph

Before using the patch, its pH is measured by soaking it with 1 milliliter of pure water for two hours at room temperature. To record the pH value, place the pH electrode on the patch's surface and allow it to adjust for one minute.

C. Percent moisture content

Following a 24-hour period in a desiccator, the patches were weighed to ascertain their percentage moisture content. Using the following formula, one may determine the percentage of moisture content:

$$\textit{Percentage Moisture content} = \textit{Initial weight} - \textit{Final weight} / \textit{Initial weight} \times 100$$

D. Percentage drug content

After being sliced into small pieces, the patches were submerged for a whole day in a phosphate buffer (PH 7.4) solution. After that, the entire solution was ultrasonically agitated for fifteen minutes. The drug concentration was determined spectrophotometrically at λmax 429 nm after filtering.

$$\textbf{Percentage Drug content} = \textbf{Absorbance of test} / \textbf{Absorbance of standard} \times 100$$

E. Folding endurance

In order to assess folding endurance, the patches were folded repeatedly in the same spot even after breaking. A patch's folding endurance is how many times it can be folded in the same spot without breaking.

F. Uniformity of weight

For every batch, the three patches were weighed, and the average weight was calculated.

G. Moisture Uptake

To maintain 84% relative humidity, the previously weighed patches were kept at room temperature in desiccators in a saturated potassium chloride solution for 24 hours. The patches were reweighed after a day, and the following formula was used to determine the percentage of moisture absorption.

$$\textbf{Percentage Moisture uptake} = \textbf{Final weight} - \textbf{Initial weight} / \textbf{Initial weight} \times 100$$

H. Percent Elongation test

When patches are subjected to external stress, they stretch, creating strain, and their elongation increases as the concentration of plasticizer increases.

$$\textbf{Percentage elongation} = \textbf{Increase in length of patch} / \textbf{Initial length of patch} \times 100$$

I. Water vapour permeability test

Water vapor permeability can be measured in an oven with regular air movement. The following formula can be used to determine the WVP.

$$\textbf{WVP} = \textbf{Amount of vapour permeated through the patch} / \textbf{Surface area}$$

J. Flatness test

Three longitudinal strips were cut from the patches; the length of each strip was measured, and the percentage constriction—0% constriction equals 100% flatness—was used to quantify the difference resulting from non-uniformity in flatness.

$$\textbf{Percentage Constriction} = \textbf{Final length of each strip} / \textbf{Initial length of each strip} \times 100$$ (V.Arunachalam1, 2023).

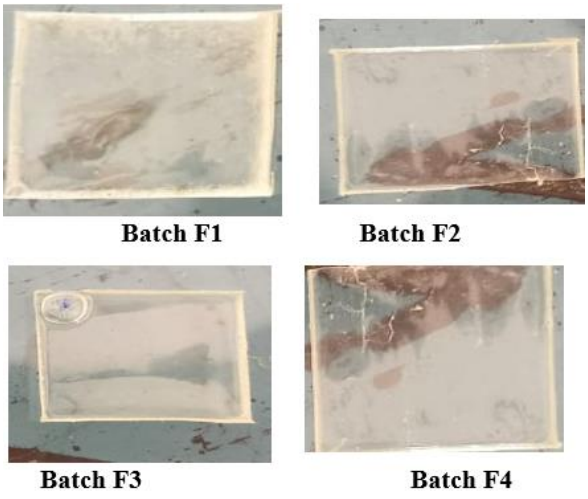

Fig no: 4 Different Batches of transdermal Patches

ANTIBACTERIAL ASSAY:

Antibacterial assay: Antimicrobials are substances that either kill or stop the growth of microorganisms like bacteria, viruses, and fungi. Antimicrobial medications that either kill microorganisms (microbicidal) or stop them from growing (microbistatic). Chemotherapy is the term used to treat microbial infections. Chemotherapeutic agents are medications that either suppress or destroy cancer cells or invasive parasites while having little to no pharmacodynamic effect on the patient. To determine whether or not the crude extracts were effective against the specific bacteria, they were initially screened for preliminary testing. The MIC test is the last step, after which only active extracts are subjected to additional testing at various concentrations. DMSO, a highly polar solvent that is harmless to microorganisms, was used to dissolve the plant extracts of Asparagus racemosus in order to retain their concentration.

I. Source of bacterial strains: - A variety of extracts were tested for antibacterial properties. Every bacterial strain was acquired from S.G.R.R. University's Department of Microbiology in Dehradun. The study was carried out at the Botany Laboratory of S.G.R.R. University's School of Basic & Applied Sciences in Dehradun.

II.Evaluation of the antibacterial potential of plant extracts: Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa were the three bacterial strains—one gram positive and two gram negative—that were used to test the antibacterial properties of the various four extracts in both dry and boiling extract form. The three bacterial strains are all harmful to humans and can result in illnesses like meningitis, food poisoning, diarrhea, fever, and pneumonia etc.

To determine whether or not the crude extracts were effective against the specific bacteria, they were initially tested for a preliminary test at a concentration of 1000 mg/ml. Additionally, allergies to common medications like streptomycin were noted. For the MIC test, only extracts exhibiting good activity were subsequently subjected to additional testing at various doses. All of the extracts were designed to dissolve in DMSO, an organic solvent that is highly polar, miscible with water and most other organic solvents, and harmless to microorganisms (Kamali, 2010).

III.Requirements for antibacterial assay

1. **Broth media: -** A liquid with nutrients for growing microorganisms is called broth. The aforementioned bacteria were grown in broth media from pure cultures kept in the Department of Life Sciences' microbiology lab at Pharmacy College Azamgarh.

a) To produce the broth, balance out the peptone, beef extract, and NaCl in an aconical flask, then add distilled water to make the capacity 500ml.

b) To dissolve all of the ingredients, the mixture was heated while being stirred.

c) Pour in 1000ml of distilled water to reach the volume.

d) Using a pH meter, the medium's pH was brought to 7.0 by adding alkali (if the pH is lower, acid may be added).

e) After covering the prepared media with a cotton plug, it was autoclaved at 121°C and 15 pounds of pressure for 15 min.

f) They let the autoclave cool. After being taken out, the soup was allowed to come to room temperature.

2. Agar Media: - The dried hydrophilic colloidal material known as agar is derived from a variety of red algae species. It dissolves when heated to 212°F (100°C) while floating in a liquid media. The medium solidifies into a gel when cooled to 110°F (43°C). In culture media, it serves as a base for the growth of bacteria and other microorganisms.

a) To prepare the agar media, combine 500ml of distilled water with the weight amount of peptone, beef extract, NaCl, and agar in a conical flask.

b) To dissolve all of the ingredients, the mixture was heated while being stirred.

c) The volume was adjusted by adding distilled water.

d) Using a pH meter, the medium's pH was brought to 7.0 by adding alkali (if the pH is lower, acid may be added).

e) The prepared media was autoclaved for 15 minutes at 121°C and 151 bs pressure while covered with a cotton plug.

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f) They let the autoclave cool. After being taken out, nutrient agar was introduced into the room.

IV.Sub-culturing of bacterial strains by slant preparation: - The growth is referred to as culture, and the food substance that microorganisms are cultivated on is termed culture medium. Slants for routine sub-culturing of the investigated bacterial strains were prepared using the Nutrient Agar Media (NAM).

Preparation of inoculums: - Place the one loop full culture in a test tube with four to five milliliters of broth, and then incubate it for two hours at 37°C. Two hours later, inoculums were ready.

Preparation of dis: - To create a disk with a diameter of 6 mm, Whatman filter paper No. 41 was punched out. After gathering all of the discs in a glass bottle, they were autoclaved for 15 minutes at 121°C and 15 pounds of pressure. Plant extract was impregnated onto the agar medium using these previously sterilized dishes in order to combat the bacterial strains under study.

V.Antibacterial assay procedure: - Five grams of peptone, three grams of beef, five grams of sodium chloride, and fifteen grams of agar were dissolved in one thousand milliliters of distilled water to create the nutritional agar medium for the antimicrobial test. On the hotplate, the media was continuously stirred to dissolve it. After that, the medium was autoclaved for 15 minutes at 15 pounds (121°C). Under aseptic conditions, it was rapidly poured into sterile petri dishes while still hot, reaching a depth of 3–4 mm, and then allowed to cool and harden. Using a micropipette, the activated bacterial culture (100µ1) was added to the agar media's solid surface. After that, it was applied using a sterile spreader to the solid agar medium surface and allowed to sit at room temperature for 15 minutes in order for absorption to take place. The agar media surface was then covered with the previously sterilized discs that had been dipped in various extracts. After that, the petri dish was incubated for 24 hours at 37°C in a BOD incubator. Using an ruler, the zone of inhibition surrounding the disc was measured in millimeters to ascertain the degree of sensitivity following incubation. All glassware was autoclaved at 15 pounds of pressure (121°C) for 15 minutes in order to sanitize it in accordance with the aforesaid process. All of the aforementioned procedures were carefully performed in laminar air flow under aseptic conditions.

Minimum Inhibitory Concertation (MIC) analysis: - The lowest concentration of an antimicrobial agent (drug) that, following an overnight incubation period, will prevent a bacterium from growing visibly is known as the Minimum Inhibitory Concentration (MIC). In diagnostic labs, minimum inhibitory concentrations are crucial for both monitoring the efficacy of novel antimicrobial drugs and verifying that microbes are resistant to them. The most fundamental laboratory assessment of an antimicrobial agent's action against an organism is typically considered to be a minimum inhibitory concentration (MIC).

In this case, only the bacterial strains that demonstrated high sensitivity in the first antibacterial tests were used to calculate the MIC values of the extracts. The active concentrated extract was serially diluted in pure DMSO for MIC analysis in order to attain a decreasing concentration range of 1000 mg/ml to 15.62 mg/ml. The MIC of the extract against the specific organism would be ascertained by employing varying concentrations of the active extract, i.e., the growth surrounding the disc to which the organism is sensitive at the lowest concentration. The zone of growth inhibition surrounding the disc was measured in order to assess the extracts' level of activity. Comparing the extract to the slandered medicine, the MIC was calculated as the concentration that demonstrated the maximum inhibition against the maximum number of bacteria at the smallest concentration. The MIC value was determined by measuring the highest zone of inhibition at the lowest concentration (Handa, 2003).

RESULT:

I. Evaluation of Herbal transdermal patches

Table.2. Physiochemical evaluation of herbal transdermal patches

S.NO	Physicochemical Evaluation	F1	F2	F3	F4
1	Thickness of Patch	0.22± 0.088g	0.2± 0.99g	0.11± 0.044g	0.17± 0.774g
2	Determination of Surface ph	4.4± 0.22	4.22± 0.092	4.33± 0.228	4.62± 0.092
3	Percent moisture content	1.99± 0.221%	0.82± 0.362%	1.32± 0.300%	1.05± 0.427%
4	% durg Content	80± 1.224%	82.6± 2.444%	84.6± 1.244%	88± 1.241%
5	Folding endurance	30.8± 0.225	35.05± 0.99	36± 0.224	31± 1.241
6	Uniformity of weight	0.88± 0.0155g	1.46± 0.0146g	1.52± 0.0147g	1.24± 0.0142g
7	percent moisture uptake	3.91± 1.332%	1.62± 0.384%	1.66± 1.014%	2.96± 1.132%
8	Percent Elongation	102.2± 0.529%	102.3± 0.526%	102.6± 0.577%	102.6± 0.526%
9	Water vapour permeability test	0.092± 1.599g/m2	0.126± 0.0002g/m2	0.133± 0.002g/m2	0.077± 0.0004g/m2
10	Flatness test	105.7± 0.667%	106.4± 2.662%	109± 4.211%	106.7± 3.412%

Table.3. Organoleptic Characteristics of Herbal Transdermal patches

S.NO.	Physical Appearance	F1	F2	F3	F4
1	Appearance	Jellified preparation	Jellified preparation	Jellified preparation	Jellified preparation
2	Colour	Light brown	Light brown	Light brown	Light brown
3	Clarity	Opaque	Opaque	Opaque	Opaque
4	Flexibility	YES	YES	YES	YES
5	Smoothness	Very Good	Very Good	Very Good	Very Good

II.Physicochemical Analysis

Table.4. Physio-Chemical Analysis

SNO.	Physicochemical Analysis	Result
1	Swelling Index	3.4 ± 0.5 ml/g
2	Angle of Repose	21° ± 5°
3	Bulk Density	0.4 to 0.6 g/ml
4	Tap Density	0.74 to 0.84 g/ml
5	Carr's Index	10.2 ± 3%
6	Hausner Ratio	1.3 to 1.5
7	Ash Valve	3.5 ± 0.5%
8	Acid Soluble Ash	1.8± 0.25 %
9	Water Soluble ash	0.83±0.25%

III.Preliminary Phytochemical Analysis:

Table.5. Phytochemicals Tested

Phytochemicals Tested	Extraction by Methanol
Alkaloids	++
Cardiac Glycosides	++
Flavonoids	++
Proteins	++
Tannins	--
Terpenoids	--
Saponins	++
Sterols	++
carbohydrate	++

Table.6. Fluorescence Analysis (UV Lamp)

CHEMICALS	VISIBLE LIGHRT	UV LIGHT (254nm)	UV LIGHT (365nm)
Distilled Water	Brown	Light Brown	Dark Brown
Conc.HCL	Red	Light Red	Black
Conc.H2SO4	Brown	Dark Brown	Black
Conc.HNO3	Brown	Brown	Dark Brown
Ethanol	Brown	Dark Brown	Dark Brown
Methanol	Brown	Dark Brown	Dark Brown
Chloroform	Light Brown	Brown	Brown
Acetone	Brown	Black	Black
Petroleum Ether	Light Brown	Brown	Brown
Sodium Hydroxide	Brown	Dark Brown	Black
Benzene	Dark Brown	Black	Black
Iodine	Brown	Black	Dark bBlack
FeCl3	Brown	Brown	Brown
Drug Power	Yellowish Brown	Light Brown	Gray White

IV.Antibacterial test

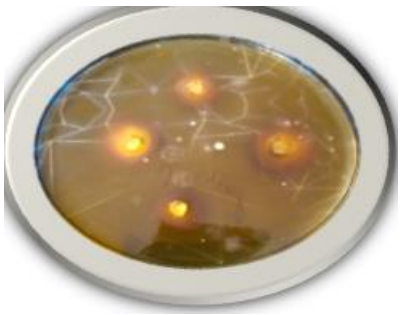

Figure 5: Staphylococcus aureus

Table no.7 Zone of inhibition

S.NO.	Bacterial strains	Zone of inhibition (in mm)				
		Acetone	Ethanol	Aqueous	Positive control	Negative control (DMSO)
1	Staphylococcus aureus	10	6	3	16	-

CONCLUSION:

The present study successfully formulated and evaluated herbal transdermal patches containing methanolic extracts of Asparagus racemosus. The prepared patches exhibited satisfactory physicochemical properties such as uniform thickness, flexibility, surface pH, and good drug content. Among the four formulations tested, Formulation F4 emerged as the most promising due to its superior drug content (88%), mechanical strength, and consistent performance in folding endurance and moisture uptake. The MIC evaluation further supported the antibacterial efficacy at lower concentrations, confirming the suitability of Asparagus racemosus as a bioactive agent in transdermal systems.

Overall, this research demonstrates the potential of Asparagus racemosus-based transdermal patches as a novel and effective alternative for topical antibacterial therapy. Future studies focusing on in vivo evaluation and clinical validation are recommended to establish its therapeutic utility and safety profile.

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