



FORMULATION AND EVALUATION OF HERBAL TRANSDERMAL PATCHES IN TREATMENT OF WOUND HEALING

1Bhumika patidar, 2Shivani solanki, 3Manoj Jaiswal, 4Lalit kushwah

1Student, 2Student, 3Associate professor, 4Assistant professor

1Charak institute of pharmacy,

2Charak institute of pharmacy,

3Charak institute of pharmacy,

4Charak institute of pharmacy

Abstract: Wound is the term which means the damage or tearing of cells and its anatomy and cell function. The wound healing is a process which involves hemostasis, inflammation, proliferation and remodeling of tissue. The proposed study was done and performed to evaluate the wound healing capacity of the herbs like psidium guajava leaf and piper betle leaf when formulated in form of transdermal patches. In this study natural wound healing was enhanced by the various phytochemicals present in guava leaf and betle leaf. The main of the study was to formulate the herbal transdermal patches for wound healing. The extract of guava leaves has been found to have antiseptic properties and can promote normal and rapid wound healing. Betle leaf extract effectively accelerates wound healing, apart out of the low risk of side effects its economically affordable and readily available in the surrounding environment. Herbal formulation is still the mainstay about 75-80% of worlds population in various country for health care because it has fewer side effects. In various study it has been seen and observed that the plants like guava and betle have the wound healing activities. Transdermal drug delivery system was introduced to overcome the difficulties of drug delivery through oral route. TDDS has wide scope in future so it involves various new approaches.

Index Terms - Wound, Transdermal, Herbal, Transdermal patches, Psidium guajava, Piper betle

I. INTRODUCTION

Herbal Medicine

The WHO has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today. Traditional medicine is the synthesis of therapeutic experience of generations of practicing physicians of indigenous system of medicine. Traditional preparations comprise medicinal plants, minerals and organic matter etc. Herbal drugs constitute only those traditional medicines which primarily use medicinal plant preparations for therapy.^[1]

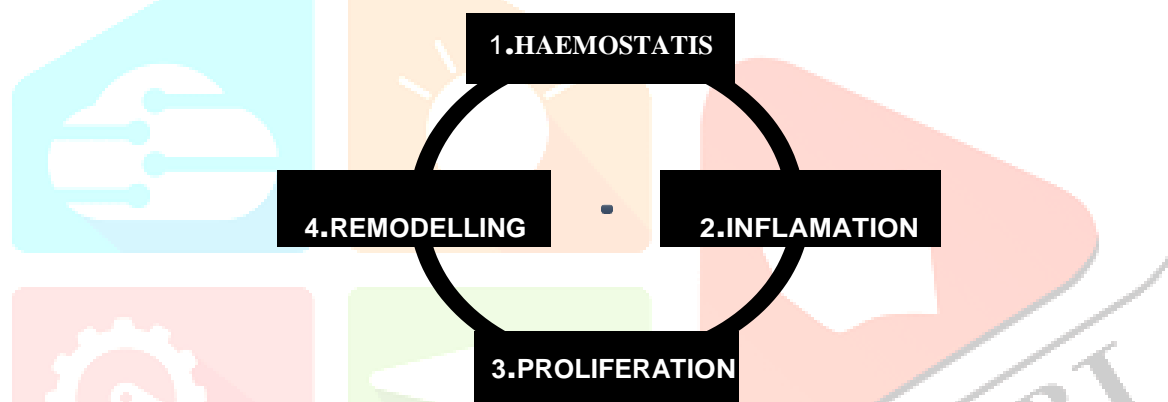
Advantages of Herbal Medicine^[2]

- They have long history of use and better patient tolerance as well as acceptance.
- Medicinal plants have a renewable source, which is only hope for sustainable supplies of cheaper medicines for the world growing population.
- Availability of medicinal plants is not a problem especially in developing countries like India having rich agro-climatic, cultural and ethnic biodiversity.
- Prolong and apparently uneventful use of herbal medicines may offer testimony of their safety and efficacy.
- Throughout the world, herbal medicine has provided many of the most potent medicines to the vast arsenal of drugs available to modern medicinal science, both in crude form and as a pure chemical upon which modern medicines are structured.

Wound Healing

Mainly, Wound is defined as the lesions on skin or rupture of skin surface which is caused by various physical or thermal trauma. (Hashemi, 2015) Skin wounds are typically of two types acute and chronic. Acute wounds are traumatic or surgical wounds that usually heal over time according to normal healing process. Acute skin wounds vary from superficial scratches to deep wounds with loss of tissue, damage to blood vessel. If the wound is large or injury is intense then there is intensive response from a body to wound. Acute wound healing is a complex process that is regulated by different types of cells and growth factors. (Nuutila, March 2014)

Steps of wound healing : -There are Four main phases of wound healing and



they are given as follows :

Haemostasis: - Haemostasis is process of the wound healing closed by clotting. Haemostasis starts when blood leaks out of the body. The first step of haemostasis is when blood vessels constrict to restrict the blood flow. Next, platelets stick together in order to seal the break in the wall of blood vessels. The haemostasis stage of wound healing happens very quickly. This establishes the fibrin provisional wound matrix and platelets provide initial release of cytokines and growth factor in the wound.

Inflammation: - Inflammation controls both bleeding and prevents infection. The fluid engorgement allows healing and repairs cells to move to site of wound. During this phase damaged cells, pathogens and bacteria are removed from wound area. This is mediated by neutrophils and macrophages which remove bacteria and denatured matrix components that retard healing and are the second source of growth factors and cytokines. Prolonged inflammation retards healing due to excessive level of protease and reactive oxygen. That destroy essential factors.

Proliferation: - Proliferative phase of wound healing is when the wound is rebuilt with new tissues made up of collagen and matrix. A new network of blood vessels must be constructed. Fibroblasts supported by new capillaries, proliferate and synthesize disorganized ECM. Basal epithelial cells proliferate and migrate over the granulation tissue to close wound surface.

Remodelling: - Also called as maturation phase. In this phase collagen is remodelled from type 3 to type 1 and wound fully closes. During maturation phase collagen is aligned along tension lines and water is reabsorbed so that collagen fibres can lie and cross link. Generally remodelling begins about 21 days after injury and can continue for a year fibroblasts and capillary densities decreases and initial scar tissue is removed and replaced By EMC that is more similar to normal skin. Cellular functions during wound healing re regulated by key

cytokines, chemokines and growth factors^[4]

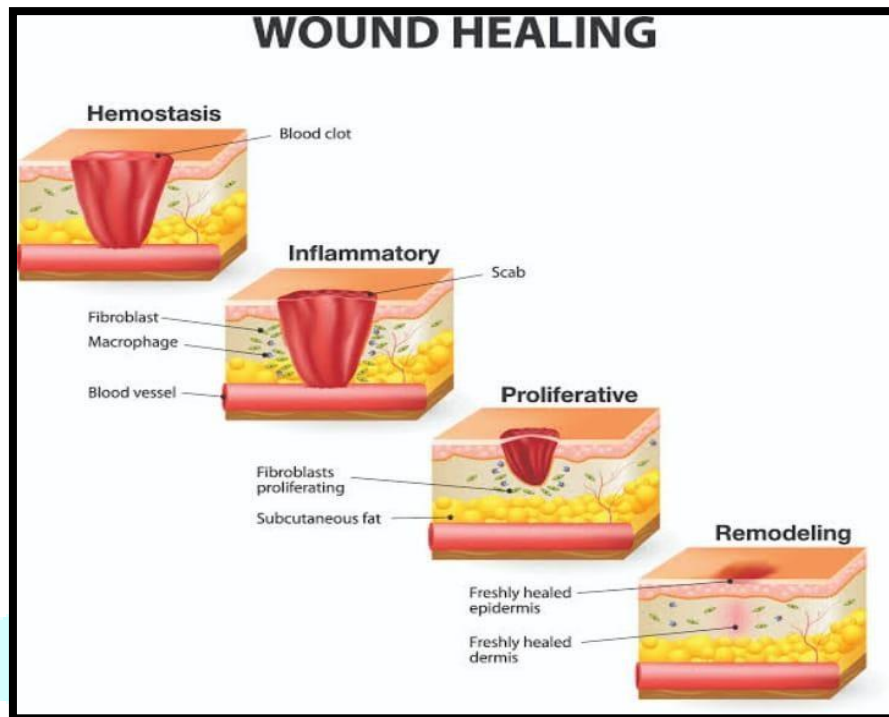


Fig.No 1 Wound healing

Transdermal drug delivery system

Transdermal drug delivery is a painless method of delivering drugs systemically by applying a drug formulation onto healthy skin. The drug initially penetrates through the stratum corneum and then passes through the deeper epidermis and dermis without drug accumulation in the dermal layer^[5,6]. TDDS is an integral part of novel drug delivery system and it was a tenth century when the skin was used as administration site for long term drug delivery. Transdermal drug delivery is the one of the most reliable as well as effective technique. Transdermal route has become one of the most successful and innovative drug delivery systems^[7]. This system was first introduced more than 20 years ago. The technology generated tremendous excitement and interest amongst major pharmaceutical companies in the 1980's and 90's. By the mid to late 1990's, the trend of Transdermal drug delivery system companies merging into larger organizations^[8]

In this approach, patches that are topically applied medications distribute medications for systemic effects at a predetermined and controlled rate. In the past, the most commonly applied systems were topically applied creams and ointments for dermatological disorders^[9]. In addition to increasing the efficacy and safety of the treatment, developing a novel delivery mechanism for current therapeutic molecules also increases patient compliance and overall therapeutic benefit to a significant extent^[10]. More recently, such dosage forms have been developed and modified in order to enhance the driving force of drug diffusion (thermodynamic activity) and to increase the permeability of the skin. These approaches include the use of penetration enhancers, supersaturated systems, hyaluronic acid, pro-drugs, liposomes and other vesicles^[11]. By boosting patient compliance and eliminating first pass metabolism, transdermal administration offers a competitive advantage over injectables and oral methods. Transdermal delivery not only allows for continuous, regulated drug administration but also removes pulsed systemic circulation for medicines with short biological half-lives, which often causes undesirable side effects. Thus, various forms of Novel drug delivery system such as Transdermal drug delivery systems, Controlled release systems, Transmucosal delivery systems etc^[12].

Transdermal patches

Transdermal patches are a mediated adhesive patch which have coating of drug and is then placed on skin to deliver the drug in the blood stream through the skin. The delivery technology like TDDS helps to enhance the convenience for patients and also increases their effectiveness and protection of drug. Transdermal patches are formulated mainly to deliver drug through skin which diffuse through various skin layer and reach systemic circulation i.e., blood

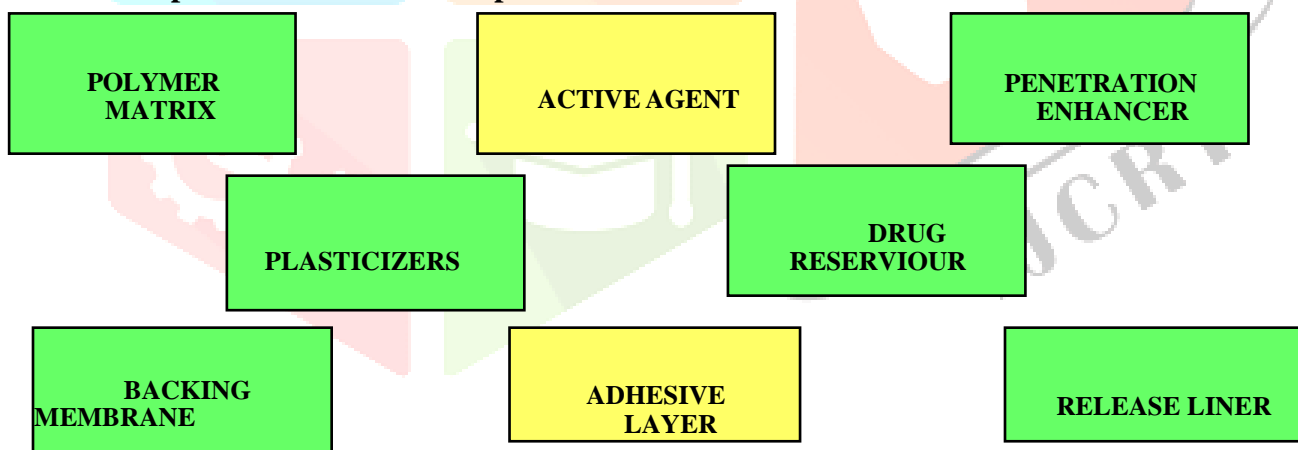
TDDS patches are defined as self-contained dosage form which when applied to the skin and deliver the drug through the skin and drug reach the systemic circulation at the controlled rate for prolonged period.

Transdermal patches can avoid or bypass the first pass metabolism which can't be bypassed by oral route. It is easy to stop the drug uptake in blood can be stopped easily by removing patches from the skin. Several synthetic drugs are prepared by transdermal patches for example Nicotine patches, Lidocaine patches, Ketoprofen patches, Diclofenac patches and many more. In the mechanism of transdermal patches skin act as a partition membrane to create barrier that control release and absorption of drug.^[13]



Fig no 2. Transdermal patch

Various components of transdermal patches :



2. Plant profile

2.1 *Psidium guajava* Linn

Table.1 *Psidium guajava* taxonomy

Kingdom	Plantae
Phylum	Magnoliophyta
Class	Magnoliopsida
Order	Myrtales
Family	Myrtaceae
Genus	<i>Psidium</i>
Species	<i>Guajava</i>



Fig.No.3 Guava leaf

2.1.1 Phytoconstituent present in *Psidium Guajava* leaf :

Alpha-pinene, beta-pinene, limonene, methanol, terphenyl acetic acid derivation, isopropyl liquor, longicyclene, caryophyllene, humulene, selinene, sardine, malic acid, nerolidol, tannin, eugenol, guajavolide and triterpenoids.

2.1.2 Pharmacological activities of *Psidium Guajava* :-

Anti-inflammatory
 Anti-amebic
 Anti-malarial
 Anti-bacterial
 Anti-cestodal
 Anti-diarrheal
 Antihyperglycemic
 Anti-stress

Cardiovascular system effects
Infectious and parasite diseases

2.1.3 Role in wound healing :-

Guava leaves have several benefits in wound healing. They have been found to be effective in cleansing wounds, removing necrotic tissue, and promoting faster healing. The use of guava leaf extract as an antiseptic has shown efficacy in wound healing compared to commercially available products. In the case of postpartum perineal wounds, the application of guava boiled water has been found to significantly reduce the duration of wound healing. Additionally, guava leaves contain phytochemical constituents such as alkaloids, flavonoids, tannins, phenols, and saponins, which contribute to their antimicrobial and antioxidant properties. These properties make guava leaves a cost-effective and natural alternative for wound care, promoting normal and rapid healing.

2.2 Piper betle

Table.2 Piper betle taxonomy

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Piperales
Family	Piperaceae
Genus	Piper
Species	Betle



Fig.No.4 Piper betle leaf

2.2.1 Phytoconstituent present in piper betle :-

Tannins, flavonoids, eugenol, hydroxychavicol, chavibetol, quercetin, diterpenes, steroids, emodins, alkaloids, saponin.

2.2.2 Pharmacological activities of Piper betle :-

Antibacterial
Antifungal
Antioxidant
Antidiabetic
Anticancer
Antimicrobial
Analgesic
Antifertility
Antihelminthic

2.2.3 Role in wound healing :-

Betel leaf extract effectively accelerates wound healing. Wounds are known as natural anatomical structure and function disturbance. Wound healing was an extremely complicated multifactor event sequence involving several cell and metabolic processes. The results indicated wound healing and repair, speeded up by the application of ointment formulation with Piper Betle leaves and stem extract which was emphasized by an ordered epidermis covering the whole wound area thickness.

3. Materials and method :-

3.1 Materials

- Plant sample
- Instruments required- Electric stirrer, Desiccator, pH meter, Weighing balance
- Apparatus required- Beakers, Measuring cylinder, Glass rod, Petri Plate
- Ingredients-

Table.3 List of Ingredients

Piper betle extract	5 ml
Guava leaf extract	5 ml
Polyethylene glycol – 400	2.5 ml
Propylene glycol	2.5 ml
HPMC	5 gm
Chloroform	8 ml
Methanol	4 ml

3.2 Method

3.2.1 Procedure for preparation of herbal plant extract :-

Guava Leaf Extract

Different components which used in Soxhlet extraction like thimble, water cooling system, and reservoir, by pass tube, siphon tube and condenser can be seen. We will take 10 mg of solid material of leaves keep in thimble which is loaded into soxhlet vessel having flask containing extractor solvent. Solvent vapor moves up to the column and floods into the chamber housing the thimble of solid. Some part of non volatile compounds dissolves in solvent. Process repeats many times until we get desired concentrated compounds in flask. Process has been done at boiling temperature of solvent and extraction has been done in 100 ml ethanol for 3.5 hours.



Fig.No5 Guava leaf extract

Piper betle leaf Extract

The leaves were freshly dried at a room temperature for 7 days then ground into powder. Cold maceration of piper betle leaf was performed by using acetone at a room temperature for 72 hours with the occasional stirring 100g of powdered piper betle leaf in 500 ml of acetone for 72 hours.

3.2.2 Procedure for formulation of transdermal patches :-

- Initially weigh the required ingredients for the formulation.
- Then add 8 ml chloroform and 4 ml methanol in the beaker and mix them properly using the electric stirrer or Magnetic stirrer.
- Then add 2.5 ml Propylene Glycol and 2.5 ml Polyethylene Glycol and again stir it continuously using electric or magnetic stirrer.
- Each medicinal plant extract i.e., 5 ml guava leaf extract and 5 ml piper betle leaf extract were added with constant stirring for 10-15 minutes.
- Now then add HPMC with constant stirring using electric stirrer. But add 1 gm. HPMC at the Time Interval of 1 minute.
- After time period of 10-15 minutes when the formulation becomes viscous then was added to the glass petri plates which were coated using the Aluminium Foil.
- Then the petri plate was placed in the Hot Air Oven at 50 degrees for certain time period for the Evaporation of solvent.



Fig No.6 Content in petri plate

4. Evaluation test

1. Organoleptic Characteristics
2. Surface pH Determination
3. Phytochemical Screening of guava leaf extract
4. Phytochemical Screening of piper betle leaf extract
5. Measurement Of Thickness of patches
6. UV Spectroscopy

7. % Moisture content

1. Organoleptic characteristics :-

In this the organoleptic properties were studied like colour, odour, appearance, and etc.

Colour: - The Colour of patches was evaluated to be whitish cream.

Texture: - The texture of the formulated patches was evaluated to be smooth and uniform.

Appearance: - The Appearance of the Formulated patches was Turbid.

Odour: - The Odour of the Formulated Patches was evaluated to be Herbal plants like.

2. Surface ph determination :-

In this evaluation test the Ph of the surface of transdermal patches was evaluated using Ph Meter.

3. PHYTOCHEMICAL SCREENING OF GUAVA LEAF EXTRACT :-

In Phytochemical screening of Tulsi extract the chemical test named as Mayer's test, Ferric Chloride test, Killer Kilani test, Benedict's test, Ninhydrin test were performed.

4. PHYTOCHEMICAL SCREENING OF PIPER BETLE LEAF EXTRACT :-

In Phytochemical screening of Aloe vera Extract the Chemical tests like Ferric Chloride test, Mayer's test, Steroid test, Lieberman's test, Ninhydrin test were Performed.

5. Measurement of thickness of patches :-

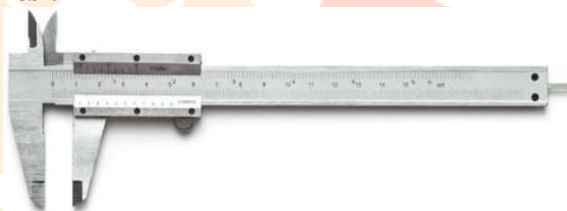


Fig No.7 Vernier calliper

6. Percentage moisture content :-

The % Moisture content was studied using Desiccator. Initially the individual patches were weighed and then kept in the desiccator containing activated silica at the Room temperature for time period of 24 hours. Then afterwards the Patches were reweighed.

$$\% \text{MOISTURE CONTENT} = \frac{[\text{INITIAL WT.} - \text{FINAL WT.}]}{\text{INITIAL WT.}} * 100$$

5. Result and Discussion

5.1 Results of Organoleptic Tests: -

Table.4 Result of organoleptic characteristics

SR.NO	CHARECTERISTIC	OBSERVATION
1.	COLOUR	Whitish cream
2.	TEXTURE	Smooth and Uniform
3.	APPEREANCE	Turbid
4.	ODOUR	Herbal Extract

5.2 Result of Ph determination: - The Ph of Formulated Patches Was found to be in Range of 5-9.



Fig.No. 8 ph determination

5.3 Phytochemical screening of guava leaf extract :-

Materials for Phytochemical Analysis-

Test-tube, conical flask, spatula, weighing balance, shaker machine

REAGENTS USED :-

10% NaOH Sodium hydroxide

5% Ferric Chloride Solution

5ml of Fehling's solution

2ml of 10% aquences hydrochloric acid

Wagner's reagent, H₂SO₄, Chloroform, ethanol. Alcoholic ferric chloride solution 5ml of 10% Ammonia solution, dilute HCl

TEST FOR FLAVONOID

3ml aliquot of the filtrated and 1ml of the 10% NaOH sodium hydroxide was mixed together, to find the possibility of flavonoid.

TEST FOR TANNINS

In this determination, a Ferric chloride solution plus 5% ferric chloride solution will be added drop by drop, 2-3mls in the solution of leave of guava extract in order to observed the appearance of Tannins.

DETERMINATION OF SAPONIN

In this test 5ml of the extract was poured in to a test tube + 5ml of water and its then shaken strongly to determine the present of saponin in the sample.

DETERMINATION OF GLYCOSIDES

2.5ml of 50% H₂SO₄ was added to 5ml of the extract in a test tube. The mixture was heated in boiling water for 15 minutes. It was cooled and neutralized with 10% NaOH, 5ml of Fehling's solution was added and the mixture was boiled.

DETERMINATION OF ALKALOIDS

About 2ml of 10% aqueous hydrochloric acid was stirred with 2ml of guava extract. 1ml was treated with a few drops of Wagners reagent and second 1ml portion was then treated simial with Mayers reagent.

TEST FOR CARDIAC GLYCOSIDES (KELLER-KILLIANI'S TEST)

A solution of herb extract with 2ml of 3.5% ferric chloride solution was added and allowed to stand for one minute. 2mls of Conc. H₂SO₄ was carefully poured down the wall of the tube so as to form a lower layer.

DETERMINATION OF TEST FOR STEROIDS (SALWOSKI)

This was carried out according to the method of Harbone 1973. 2ml of the extract was dissolved in 2ml of chloroform. 2ml of sulphuric acid was carefully added to form lower layer.

TEST FOR SAPONIN GLYCOSIDES

To 2.5ml of the extract was added 2.5ml of Fehling's solution,

TEST FOR BASALMS

9.5ml of the extract was mixed with equal volume of 90% ethanol, 2 drops of alcoholic ferric chloride solution was added to the mixture..

TEST FOR ANTHRAQUINONES

2ml of each plant extract was shaken with 10ml benzene, and 5ml of 10% ammonia solution was added. The mixture was shaken in order to obtained the coulor of antraquinonesand.

TEST FOR VOLATILE OILS

1ML OF THE FRACTION WAS MIXED WITH DILUTE HCL. A WHITE PRECIPITATE WAS NOT FORMED, THIS INDICATED THE ABSENCE OF VOLATILE OILS.

RESULT

The phytochemical screening of Guava extractrevealed the presence of saponins, alkaloids, volatile oil, steroids, balsmas, saponin glycosides, flavonoids, tannins, and anthraquinone While glycosides and cardiac glycosides were absent as stated in table

Table.5 phytochemical test of guava leaf extract

PHYTOCHEMICALS	RESULT
Tannins	+
Saponins	+
Steroids	+
Saponin glycosides	+
Glycosides	-

Flavanoids	+
Alkaloids	+
Cardiac glycosides	-
Volatile oil	-
Anthraquinone	+
Balsmas	+

+ presence of constituents

-Absence of constituents

5.4 Phytochemical screening of piper betle leaf:-

Phytochemical analysis:

Test for Alkaloids: To the extract added 1% HCl and 6 drops of Mayer's reagent and Dragendroff's reagent. An organic precipitate indicated the presence of alkaloids in the sample.

Test for Flavonoids: 5 ml of dilute ammonia solution were added to a portion of aqueous filtrate of each plant extract followed by addition of conc. H_2SO_4 . A yellow coloration is observed which confirms the presence of flavonoids and it disappears on standing .

Test of glycosides:

Dissolve small amount of an alcoholic extract of the fresh or dried material in one ml of water. Add a few drops of aqueous NaOH solution. Yellow color indicates the presence of glycoside .

Test for Steroids:

2 ml of acetic anhydride was added to 0.5gm of ethanolic extract of each sample with 2 ml of H_2SO_4 . The color change from violet to blue or green indicated the presence of steroids

Test for Tannins:

5 ml of extract was added to few drops of 1% lead acetate. A yellow precipitate indicated the presence of tannins .

Test for Terpenoids:

5 ml of each extract was added to 2 ml of chloroform and 3 ml of conc. H_2SO_4 to form a monolayer of reddish brown coloration of the interface was showed to form positive result for the terpenoids .

Test for Saponins:

The extract with 20 ml of distilled water was agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam indicated the presence of saponins .

RESULT**Table.6 phytochemical screening of piper betle leaf**

PHYTOCHEMICAL	RESULT
Alkaloids	+
Glycosides	+
Cardiac glycosides	+
Anthraquinone glycosides	+
Terpenoids	+
Flavonoids	+
Tannins	+
Saponins	+

+ presence of phytochemical

- absence of phytochemical

5.5 Result of Thickness Measurement: - The Thickness of Formulated transdermal patches were evaluated to be **0.22mm** using vernier Calliper. This Thickness was evaluated by measuring the average Thickness from three Sites of the patches.

5.6 Result For %Moisture Content: -

Here,

Initial weight=0.6gm and Final weight=0.3gm so By Using the formula of %MC

%Moisture content= $[0.6-0.3]/0.6*100=50\%$

Hence, the %Moisture Content was evaluated to be **50%**

6. Conclusion

The Transdermal patches with the incorporation of herbal extract of psidium guava leaf and piper betle leaf were formulated. As Earlier it was discussed that now in the emerging world there is more demand to the Herbal formulation. In the latest research studies, it has come to see that there is a wide scope for Implementation of Novel Drug Delivery System. As we all are known to some overpowering benefits of Novel drug delivery system over the traditional drug delivery system. As it is in concern with the drug delivery through the skin the Transdermal Drug delivery system has an effective benefit over the topical method of drug delivery. The advancement in drug delivery system is allowing wide range of drugs to be administered through transdermal drug delivery system. As discussed earlier there are lots of differences between topical and transdermal system which leads the selection of drug product very crucial and complex. The Herbal transdermal Patches including guava leaf and piper betle leaf were aimed to heal the wound. The Evaluation studies states that the patches have the optimum thickness and is within the suitable range of Ph. In the various research it's seen that transdermal drug delivery system has great scope in future for developing drug delivery system in NDDS. Transdermal drug delivery system is widely accepted now-a-days because it causes the drug penetration

through skin layers and reach systemic circulation without causing any damage to skin or rupturing it. TDDS also benefits for controlled release of drug for prolonged period of time. More research and innovation will bring the wide acceptance in the use of various other transdermal drug delivery system like iontophoresis, Ultrasound technology, Med Tat etc.

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