FORMULATION AND EVALUATION OF TRANSDERMAL PATCHES USING ISOLATED SOLASODINE FROM *SOLANUM SURATTENSE* FOR ANTI- INFLAMMATORY, ANALGESIC AND ANTIPYRETIC ACTIVITY

A Dissertation submitted to

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In partial fulfillment of the requirements for the award of degree of

MASTER OF PHARMACY IN PHARMACOGNOSY

Submitted by

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Under the guidance of

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CERTIFICATE

This is to certify that the dissertation entitled "**FORMULATION AND EVALUATION OF TRANSDERMAL PATCHES USING ISOLATED SOLASODINE FROM *SOLANUM SURATTENSE* FOR ANTI-INFLAMMATORY, ANALGESIC AND ANTI PYRETIC ACTIVITY**". Submitted by Reg.no.261220653 in partial fulfillment of the requirements for the award of the degree of **Master of pharmacy in Pharmacognosy** by The Tamil Nadu Dr.M.G.R. Medical university, Chennai, is a bonafide work done by her during the academic year 2013-2014 under the guidance of **Dr. R.Radha, M.Pharm., Ph.D.,** Department of Pharmacognosy, Madras Medical College, Chennai 600003.

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DEDICATED TO MY BELOVED PARENTS AND GOWRISHANKAR

1. INTRODUCTION

"The art of healing comes from nature and not from physician. Therefore, the physician must start from nature with open mind"
- Paracelsus
"For every drug that benefits a patient, there is a natural substance that can achieve the same effect"
- Pfeiffer's law

1.1 HISTORY OF USE OF TRADITIONAL HERBAL MEDICINES

By the definition 'traditional' use of herbal medicine implies substantial historical use and this is certainly true for many products that are available as 'traditional herbal medicines'. In many developing countries, a large proportion of the population relies on traditional practitioners and their armamentarium of medicinal plants in order to meet health care needs. Although modern medicine may exist side by said with such traditional practice, herbal medicines often maintain their popularity for historical and cultural reasons. Such products have become more widely available commercially [1].

Herbal medicine has been a backbone for revitalizing human body systems from early stages of human history. Plants with the major healing powers are almost from ancient civilizations like the Egyptian, The Chinese, The Indian, Roman and also Greek.

1.2 INDIAN TRADITIONAL MEDICINE

Herbal medicine is also called phyto-medicine using a plant's seeds, berries, roots, leaves, bark or flowers for medicinal purposes. Herbalism has a long tradition of use outside of conventional medicine. It is becoming more main stream as improvements in analysis and quality control along with advances in clinical research and the value of herbal medicine in the treatment and prevention of disease.

Ayurveda is a medicinal system primarily practiced in India that has been known for near 5000 years. It includes diet and herbal remedies, while emphasizing the body, mind and spirit in disease prevention and treatment.

Various pharmaceutical processes are prescribed in Ayurveda to achieve the following [2]:

- Isolation of active constituent of the drug
- Easy administration of the drug
- Easy digestion and assimilation
- Better preservation
- Better therapeutic tolerance

Many of the pharmaceuticals currently available have a long history of use as herbal remedies, including opium, aspirin, digitalis, and quinine. The World Health Organization (WHO) estimates that 80 percent of the populations of some Asian and African countries presently use herbal medicine for some aspect of primary health care. In comparison, herbal medicines can be grown from seed or gathered from nature for little or no cost than pharmaceuticals. The use of, and search for, drugs and dietary supplements derived from plants have accelerated in recent years. Pharmacologists, microbiologists, botanists, and natural-products chemists are combing the Earth for phytochemicals and leads that could be developed for treatment of various diseases. Among the 120 active compounds currently isolated from the higher plants and widely used in modern medicine today, 80 percent show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived. More than two thirds of the world's plant species - at least 35,000 of which are estimated to have medicinal value - come from the developing countries. At least 7,000 medical compounds in the modern pharmacopoeia are derived from plants. In many medicinal and aromatic plants (MAPs) significant variations of plants characteristics have been ascertained with varying soil traits, and the selective recovery and subsequent release in food of certain elements have been demonstrated. Great attention must be paid to choose soil and cropping strategies, to obtain satisfactory yields of high quality and best-priced products, respecting their safety and nutritional value.

1.3 TRADITIONAL DOSAGE FORMS OF AYURVEDIC MEDICINES [2]

The dosage forms depend on the source of the drug and whether it will be used as such or processed further. The methods for refining crude drugs from plants are different from those for compound formulations.

COMPOUND FORMULAIONS:

Some of the compound formulations in Ayurveda as follows,

- Choornam
- Arishtas and Asavas
- Avalehya, Leha and Paaka
- Bhasmas
- Kshara
- Gutikas or Gulikas
- Thailam or Thailas

1.3.1 ADVANTAGES AND DISADVANTAGES OF AYURVEDIC FORMULATIONS [2]

Advantages:

- Global reach for products: Mass production of ayurvedic products has led to their wide spread availability. Drugs from flora and fauna limited to certain zones can be delivered globally.
- Creative marketing strategies: Because of dynamic marketing approaches, certain ayurvedic medicines have become a household name. The promotion and popularization of ayurvedic products has resulted in greater interest in the system as a whole.
- Scope for research and development: Due to the growing competition, the industry has opted to invest in research and development of new products and processes. This has greatly improved the quality and potential of ayurvedic medicines.

Disadvantages:

- Lack of standardization: many of the processes described in ancient literature are not designed for production on an industrial scale. Due to a lack of standardization parameters, the initial phase of transition was tough.
- Pressure on raw material resources: the spurt in demand has placed greater pressure on the raw material resources. This has led to environmental degradation, extinction and loss of biological 'sinks'. It also resulted in sub-standard materials being used as inputs.

1.4 ADVANTAGES OF HERBAL MEDICINES [1,4]

- Herbal medicines are cost effective and less expensive than all allopathic medicines.
- Herbal medicines are considered to be more effective than allopathic medicines for certain ailments.
- Herbal medicines do not have any side effects, as they are free from chemicals. They are also milder than allopathic medicines.
- Herbal medicines are very effective in curing various digestive disorders like colitis, indigestion, peptic ulcers, and irregular bowel movements.
- These type of medicines are best for the people who are allergic to various types of drugs
- Herbal medicines are also effective in boosting the mental health.
- Most of the ailments related to blood circulation like high blood pressure, varicose ulcers, and many others can be controlled through herbal medicines.
- They can also be used to reduce weight by regulating appetite.

Though herbal medicines have many advantages, it has some main disadvantages also.

DISADVANTAGES

- The main disadvantage is that herbal medicines take too much time to act. The entire process is very slow.
- Large dose is required when compared to allopathic medicine to treat a particular disease.

Now days, to overcome these disadvantages the herbal medicines are formulated using novel drug delivery system.

1.5 NOVEL DRUG DELIVERY SYSTEM [5]

NDDS is an advanced drug delivery system which improves drug potency, controls drug release to give a sustained therapeutic effect, provides greater safety and targets drug specifically to a desired tissue.

1.5.1 NDDS MAY BE ACHEIVED BY SEVERAL SYSTEMS [8]

- Oral drug delivery systems and materials
- Parenteral and implant drug delivery system.
- Pulmonary and nasal drug delivery
- Trans- mucosal drug delivery.
- Transversal and topical drug delivery.
- Delivery of proteins and peptides.

1.6 TRANSDERMAL DRUG DELIVERY SYSTEM

Transdermal therapeutic systems are defined as self-contained, discrete dosage forms which, when applied to the intact skin, deliver the drug, through the skin, at a controlled rate to the systemic circulation

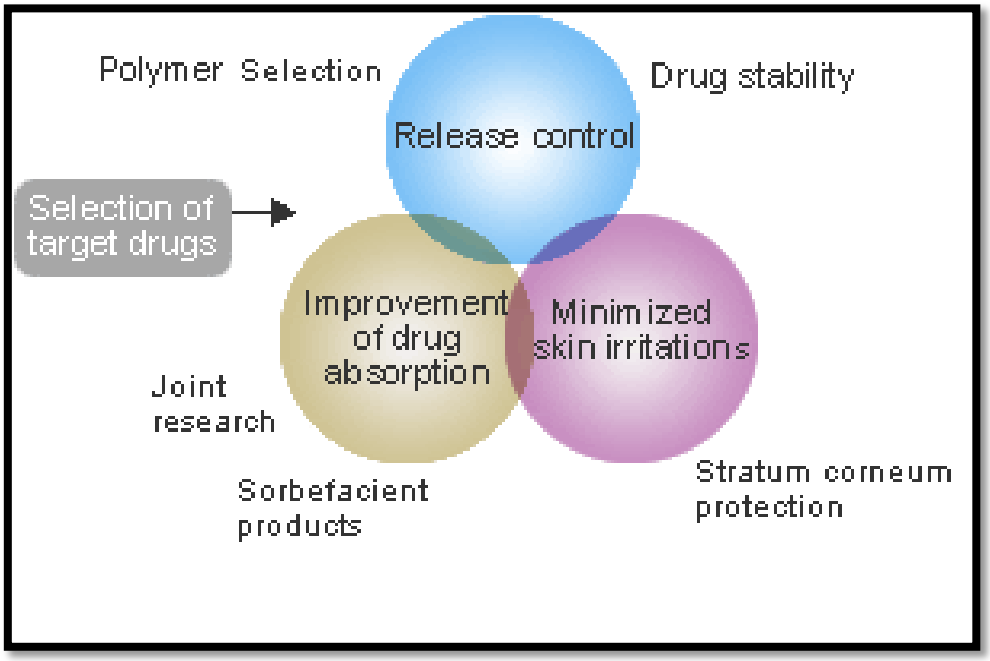

Fig 1: Advancement in drug delivery system

ADVANTEGES [6]

1. Transdermal delivery can increase the therapeutic value of many drugs by avoiding specific problems associated with the drug eg., gastro-intestinal irritation, low absorption, decomposition due to hepatic "first pass" effect, formation of metabolites that cause side effects, short half-life necessitating frequent dosing etc.
2. Due to the above advantage, it is possible that an equivalent therapeutic effect can be elicited via transdermal drug input with a lower daily dose of the drug than is necessary.
3. The simplified medication regimen leads to improved patient compliance and reduced inter and intra patient variability

4. At times maintenance of constant drug concentration within the biophase is not desired. Application and removal of transdermal patch produce the optimal sequence of pharmacological effect.
5. Self administration is possible with these systems.
6. The drug input can be terminated at any point of time by removing transdermal patch.

LIMITATIONS

1. Skin irritation or contact dermatitis due to the drug, excipients and enhancers of the drug used to increase percutaneous absorption.
2. The barrier function of the skin may changes from one place to another on the same person, from person to person and with age.
3. If the drug dose required for therapeutic value is more than 10mg/day, the transdermal delivery will be very difficult. Daily dose less than 5mg/day are preferred.

Fig 2: Transdermal patches

Fig3: Easy self administration

Fig 4: Convenience in handling

1.6.1 APPROACHES USED IN DEVELOPMENT OF TRANSDERMAL DRUG DELIVERY SYSTEM [8]

Though the notion of using the skin as a route of drug administration had been conceived earlier, it was only in 1981, the technology for the rate - controlled administration of drugs through intact skin become available.

All such transdermal dosage forms have a basic structure comprising of many layers, each having a specific function. Farthest from the skin, when the system is in place, is a backing layer, preventing wetting of the system during use. The second layer is a reservoir that supplies a continuous quantum of drug for the predetermined functional life time of the system. Next to the reservoir is the rate control polymeric membrane which regulates the rate of drug during a predetermined time interval. The drug so delivered diffuses through the skin and enters the systemic circulation. The rate of drug release from transdermal system is normally much greater than the amount than the skin can possibly absorb. Hence, even if there are variations in skin permeability, a constant rate of drug input into the circulation is achieved.

Four different approaches have been utilized to obtain transdermal drug delivery systems

- Membrane permeation - controlled systems.
- Adhesive dispersion – type system.
- Matrix diffusion – controlled system.
- Microreservoir type or microsealed dissolution controlled systems.

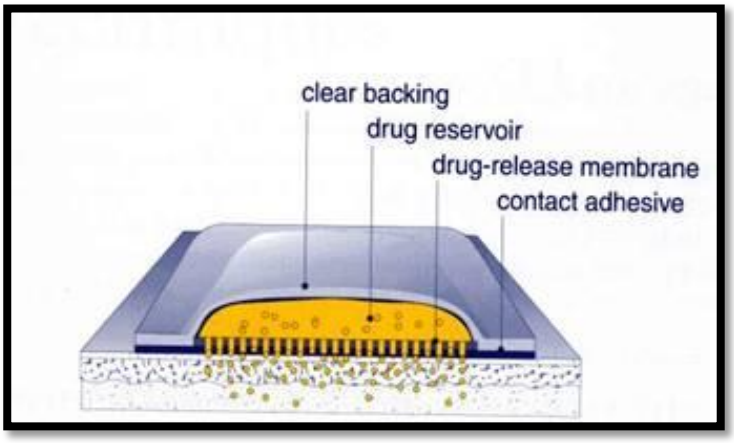

Fig 5: Components of transdermal drug delivery system

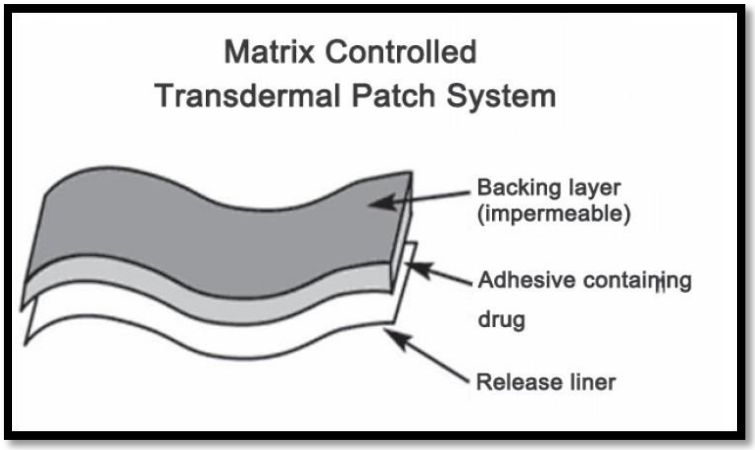

Fig 6: Matrix controlled type TDS

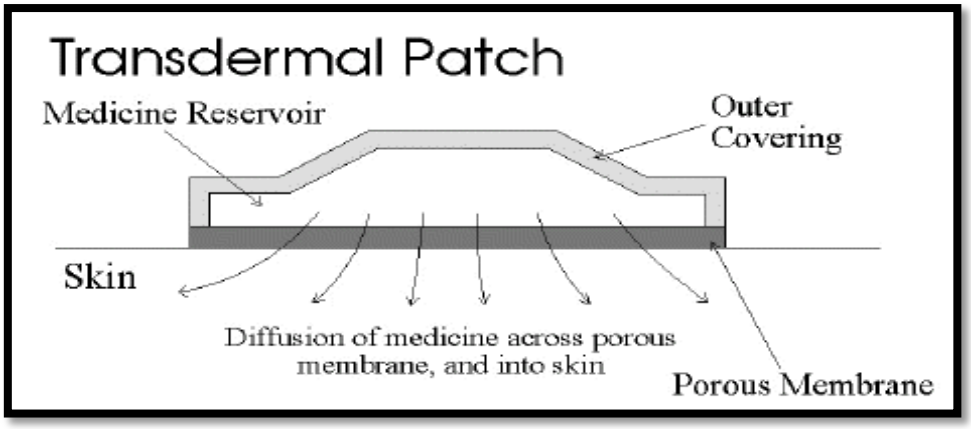

Fig 7: Reservoir type TDS

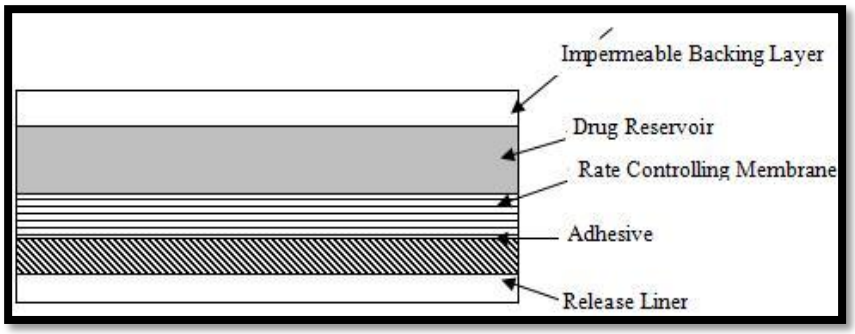

Fig 8: Cross section of reservoir type TDS

1.7 ANTI-INFLAMATORY, ANALGESIC AND ANTI-PYRETIC ACTIVITY [9]

Essential to survival of organisms is their ability to get rid of damaged or necrotic tissues and foreign invaders, such as microbes. The host response that accomplishes these goals is called inflammation. This is fundamentally a protective response, designed to rid the organism of both the initial cause of cell injury (eg., microbes, toxins).

Inflammation is a complex reaction in tissues that consist mainly of responses of blood vessels and leukocytes.

Inflammation may be acute or chronic, depending on the nature of stimulus and the effectiveness of the initial reaction in eliminating the stimulus or damaged tissues. Acute inflammation is rapid in onset (typically minutes) and is of short duration, lasting for hours or few days; its main characteristics are the exudation of fluid and plasma proteins (edema) and the emigration of leukocytes, predominantly neutrophils. When acute inflammation is successful in eliminating the offenders the reaction subsides, but if the response fails to clear the invaders it can progress to a chronic phase. Chronic inflammation may follow acute inflammation or be insidious in onset. It is of longer duration and is associated with the presence of lymphocytes and macrophages, the proliferation of blood vessels, fibrosis and tissue destruction.

Inflammation is terminated when the offending agent is eliminated. The reaction resolves rapidly, because the mediators are broken down and dissipated and the leukocytes have short life spans in tissues. In addition, anti- inflammatory mechanisms are activated that serves to control the response and prevent it from causing excessive damage to the host.

Inflammation is a body defense reaction to eliminate or limit the spread of an injurious agent and is characterized by four cardinal signs,

- Redness (rubor)
- Swelling (tumor)
- Heat (calor)
- Pain (dolor)

ACUTE INFLAMMATION

Acute inflammation is a rapid host response that serves to deliver leukocytes and plasma proteins, such as antibodies, to sites of infection or tissue injury. Acute inflammation has three major components:

1. Alterations in vascular caliber that lead to an increase in blood flow
2. Structural changes in the microvasculature that permit plasma proteins and leukocytes to leave the circulation.
3. Emigration of leukocytes from the microcirculation, their accumulation in the focus of injury, and their activation to eliminate the offending agent.

TERMINATION OF ACUTE INFLAMMATORY RESPONSE

It is predicable that such a powerful system of host defense, with its inherent capacity to cause tissue damage, needs tight control to minimize the damage. In part, inflammation declines simply because the mediators of inflammation are produced in rapid bursts, only as long as the stimulus persists, have short half-lives, and are degraded after their release. Neutrophils also have short half-lives in tissues and die by apoptosis within a few hours after leaving the blood. In addition, as inflammation develops the process also triggers a variety of stop signals that serve to actively terminate the reaction. This active termination mechanism include a switch in the type of arachidonic acid metabolite produced, from pro inflammatory leukotrienes to anti-inflammatory lipoxins; liberation of anti-inflammatory cytokines, including transforming growth factor-β (TGF-β) and IL-10, from macrophages and other cells; he production of anti-inflammaory lipid mediators, called resolvins and protectins, derived from polyunsaturated fatty acids; and neural impulses (cholinergic discharge) that inhibit the production of TNF in macrophages.

CHRONIC INFLAMMATION

Chronic inflammation is inflammation of prolonged duration (weeks or months) in which inflammation, tissue injury and attempts at repair coexist, in varying combinations. It may follow acute inflammation, as described earlier, or chronic inflammation may begin insidiously, as a low grade, smoldering response without any manifestations of an acute reaction. This latter type of chronic inflammation is the cause of tissue damage in some of the most common and disabling human diseases, such as rheumatoid arthritis, atherosclerosis, tuberculosis and pulmonary fibrosis. It has also been implicated in the progression of cancer and in diseases once thought to be purely degenerative, such as Alzheimer disease.

MORPHOLOGIC FEATURES

In contrast to acute inflammation, which is manifested b vascular changes, edema and predominantly neutrophilic infiltration, chronic inflammation is characterized by:

1. Infiltration with mononuclear cells, which include macrophages, lymphocytes, and plasma cells.
2. Tissue destruction, induced b the persistent offending agent or b the inflammatory cells.
3. Attempts at healing by connective tissue replacement of damaged tissue, accomplished by proliferation of small blood vessels (angiogenesis) and in particular, fibrosis.

Analgesia: The pain sensitizing mechanism is induced by bradykinin, ILs and TNFα etc. Therefore drugs that are useful in inflammation are more effective in inflammation associated with pain also.

2. REVIEW OF LITERATURE

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3. PLANT PROFILE

Solanum surattense [3,10]

Introduction

Solanum surattense, an herb grass as wild plant in many parts of India. Fruits and leaves are used in the treatment of various ailments as traditional folk medicine.

Vernacular Names

English	:	*Solanum surattense*
Hindi	:	Kantkari
Tamil	:	Kantankatiri
Other names	:	Chotic katheri, kateli, Bhatkatiya

Macroscopy

Leaves

Petiolate, exstipulate, ovate- oblong or elliptic, sinuate or sub-pinnatified, sub-acute hairy; 4-12.5 cm long and 2-7.5 cm wide; green; veins and midrib full with sharp prickles; odour and taste not distinct.

Fruit

Berry, globular, measuring 0.8-1 cm in diameter, surrounded by persistant calyx at base unripe fruits variegated with green and white strips; ripe fruit shows different yellow and white shades.

Microscopy

Leaves

1. Petiole – transverse section of petiole shows circular to wavy outlines; epidermis single layered, covered externally by thick cuticle; hypodermis consists of 3-4 layers of collenchymatous cells; one large- crescent- shaped, bicollateral, central vascular

bundle and two small lateral bundles present; rest of tissue of petiole composed of polygonal, angular, thin-walled, parenchymatous cells; epidermis shows mostly stellate and rarely uni to tricellular hairs.

2. Midrib – transverse section of midrib shows a biconvex structure; epidermis on either side covered externally by thick cuticle; below epidermis 3-4 layers of collenchyma present; style composed of crescent shaped, bicollateral, central vascular bundle and two small lateral vascular bundles; rest of tissue composed of thin-walled, parenchyma, some stellate hair present on epidermis.

3. Lamina – transverse section shows dorsiventral structure; epidermis on either side, wavy in outline, covered externally by thick cuticle; on upper side mesophyll composed of a single layered palisade and 4-6 layers of loosely arranged spongy parenchyma; some stellate hairs (4-8 armed) present on both sides of epidermis; anisocytic stomata present on both surface; vein-islet number 46-80 on lower epidermis (mean 63), 61-80 on upper epidermis (mean 70); stomatal index 20-25 (mean 22.5) on lower epidermis, 14-24 (mean 19) upper epidermis, palisade ratio 1.74 (mean 2.85).

Fruit

Transverse section of mature fruit shows single layered epidermis, covered externally by thin cuticle; 1-2 layers of collenchyma present below epidermis; mesocarp composed of thin walled, oval to polygonal cells; some fibre vascular bundles present scattered; seed consist of thick-walled radially elongated testa, narrow endosperm with embryo; some cells of endosperm contain oil globules.

Fig 11: Leaves and berries of *Solanum suretense*

Description and Distribution [13]

Solanum surattense is an annual herbaceous plant comprising 90 genera and 2000 – 3000 species. In India, it is mainly grown in Uttar Pradesh, Bihar, Uttaranchal, Punjab, West Bengal, Assam and other North-Eastern states. It is generally grown in March – April and bears fruits in May – June. It grows on all kinds of soil but does well on dry and hot temperature. It is very prickly diffuse, bright green perennial herb, 2-3m height; stems zigzag; prickles compressed, straight , yellow and shining; leaves 5-10 by 2.5-5.7cm ovate or elliptic, sinuate or sub pinnatified, hairy on both sides , petiole prickly flowers are small, in extra axillary few flowered cymes, corolla is purple, lobes deltoid, hairy outside. Fruits are 1.3cm diameter berry yellow or white with green veins, surrounded by enlarged calyx.

S.surattense fruits yield Solano carpi dine and a sterol, carp sterol. Root is one of the constituents of dasamlasava. Seeds are used as Diuretic. Juice of berries is reported to be useful in sore throat. A decoction of plant is used in gonorrhea and it also said to promote contraception in females. Kantakari is reported useful in Kasa Roga (cough) and also in Tamakwasa (bronchial asthma). In Chhattisgarh, it is considered as a most valuable herb for traditional healers in treatment of over 100 common diseases alone or in combination with other local exotic herbs. According to Ayurveda, it is bitter, appetizer, laxative, anthelmintic, stomachic and useful in bronchitis, asthma, fever, lumbago, pains, piles (especially in bleeding piles), thirst, urinary and heart diseases.

Fig 12: Herb of *Solanum surettense*

Chemical Constituents [11]

Plant contains alkaloids, sterols, saponins, flavonoids and their glycosides and also carbohydrates, fatty acids, amino acids etc.

In India, glycoalkaloid content of fruits collected from Jammu and Kashmir is reported to be 305% (total alkaloid, 1.1%). Plant samples collected from Kolkata contained solasodine (0.0287%). Plant contains diosgenin seeds yield greenish yellow, semi-drying oil (19.3%) with a characteristic odour. Unsaponofiable matter of fruits contains two sterols, one of which is carpesterol.

Pharmacological Action [10]

Stimulant, expectorant, diuretic, laxative, febrifuge used in the treatment of cough, bronchitis, asthma for dislodging tenacious phlegm, and also used against rheumatic, enlargement of liver and spleen, vomiting, difficult urination, bladder stones, skin disease. Fruits are used as an adjuvant for promoting contraception.

Dosage

Whole plant – 20 to 30kg for decoction.

4. AIM AND OBJECTIVE

Non-steroidal anti-inflammatory drugs (NSAIDS) are widely used in the treatment of acute and chronic inflammation, pain and fever. But the greatest disadvantage in presently available synthetic drugs is that they cause gastrointestinal irritation and reappearance of symptoms after discontinuation [5,46].

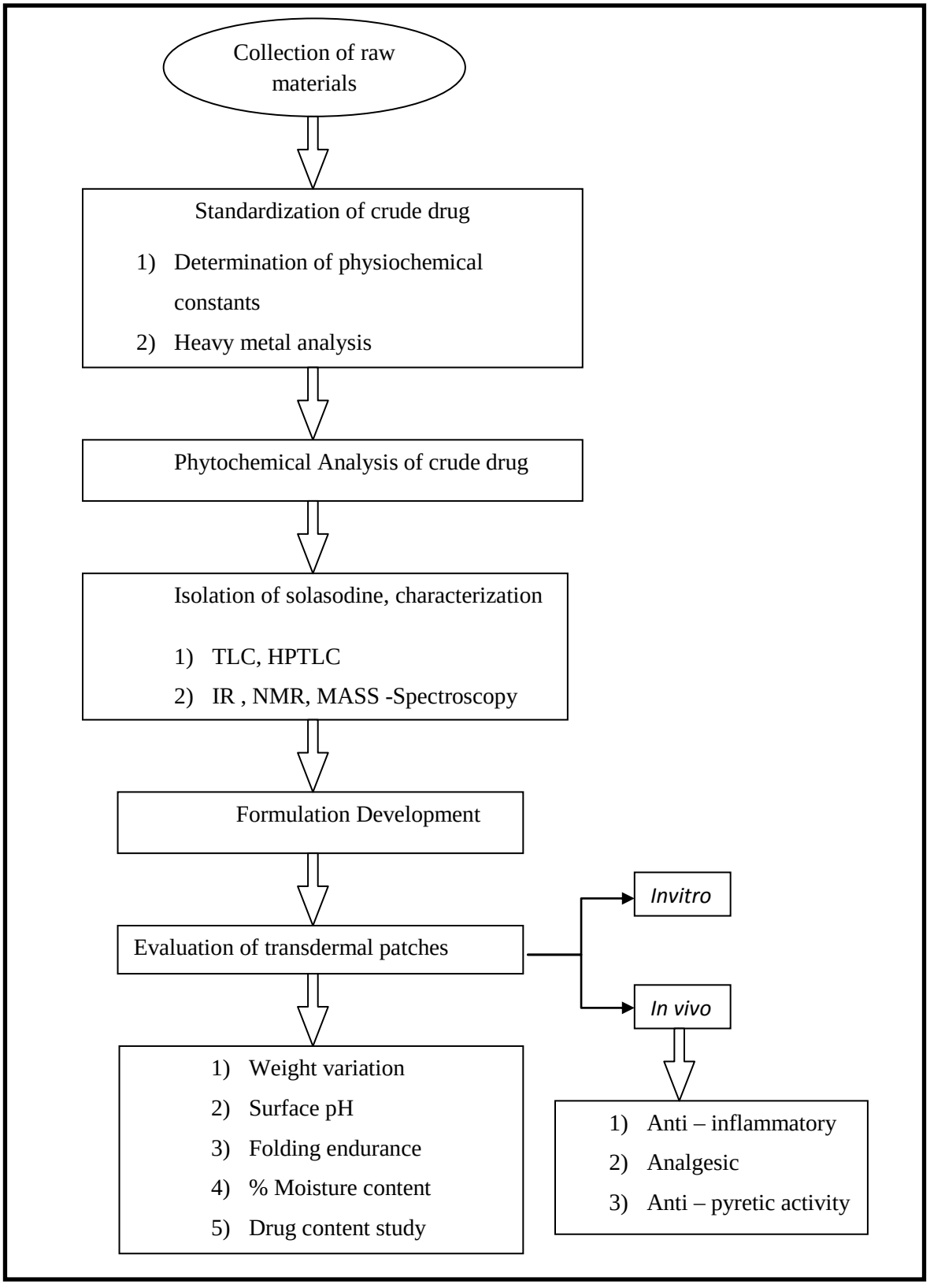
Therefore, there is a dire need for screening and development of novel and better anti-inflammatory, analgesic and anti-pyretic drug that will be effective with better safety profile. Indigenous medicinal plants could be a logical source to find these.

Solanum surattense has many therapeutic uses mentioned in Ayurveda, especially Anti-inflammatory property [3]. This is mainly due to the alkaloid solasodine which is found in leaves and berries of the plant *S.surattense*. This plant is available throughout India and hence I intended to carry out my work to formulate and evaluate the transdermal patches from isolated solasodine of *Solanum surattense* for anti-inflammatory, analgesic and anti-pyretic activity.

Objectives of the study include

- To standardize the crude drug used for the Isolation.
- Isolation of solasodine from *S.Surattense*.
- Characterization of isolated compound.
- Formulation of transdermal patches using isolated compound of solasodine.
- Standardization and evaluation of transdermal patches.
- *Invitro* evaluation of Transdermal paches.
- Skin irritation test of Transdermal patches in rabbits.
- *Invivo* evaluation for anti-inflammatory, anti-pyretic and analgesic activity.

5. PLAN OF WORK



6. MATERIALS AND METHODS

6.1 STANDARDIZATION OF RAW MATERIAL

COLLECTION OF PLANT MATERIAL

The fresh leaves and berries of *Solanum surattense* were collected in the month of July – September from local areas of Salem and authenticated by Dr.P.Jayaraman, Director, Plant anatomy research center, Tambaram, Chennai.

The part of plant was shade dried at room temperature and reduced to a coarse powder. It is used for standardization and isolation purpose.

6.1.1 DETERMINATION OF PHYSIOCHEMICAL CONSTANTS [24]

a. DETERMINATION OF MOISTURE CONTENT

The loss on drying test is important when the herbal substances are known to be hygroscopic. An excess of water in medicinal plant material will encourage microbial growth, the presence of fungi, insects and deterioration. In modern pharmaceutical technology, the water content provides information concerning the shelf life and quality of the drug.

About 10g of the drug (without preliminary drying) was placed after accurately weighed (within 0.01g) in a tarred evaporating dish and dried at 105°C for 5 hours and weighed. The drying was continued and weighed, until the difference between two successive weighing corresponds to not more than 0.25 percent at one hour interval. Constant weight is reached when two consecutive weight after drying for 30 minutes and cool for 30 minutes in a desiccators show not more than 0.01g difference.

$$\text{Moisture content (percentage)} = \frac{\text{Final weight of the sample}}{\text{Initial weight of the sample}} \times 100$$

b. ASH VALUE

The ash content of crude drug is generally taken to be the residue remaining after incineration. It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may include inorganic matter added for the purpose of adulteration. Ash value varies within narrow limits in case of the individual drug but varies considerably in case of polyherbal formulation. Hence the ash value determination furnishes a basis of judging the identity and cleanliness of a drug and gives information relative to is adulteration with inorganic matter. Ash standards have been established for a number of official drugs usually these standards set a maximum limit on the total ash or on the acid insoluble ash permitted.

b.1 DETERMINATION OF TOTAL ASH

The total ash method is designed to measure the total amount of material remaining after ignition. This includes both "physiological ash", which is derived from the plant tissue itself, and "Non – physiological ash", which is the residue of the extraneous matter (e.g. Sand and soil) adhering to the plant surface.

About 2g of substance of the ground drug was incinerated in a tarred silica crucible at a temperature not exceeding 450°C until free from carbon, cooled and weighed. If a carbon free ash cannot be obtained in this way exhausts the charred mass with hot water, the residue was collected on an ash less filter paper, incinerate the residue and filer paper and ignited at a temperature not exceeding 450°C. The percentage was calculated with reference to the air-dried drug.

b.2 DETERMINATION OF ACID INSOLUBE ASH

For acid insoluble testing, the total ash was boiled for 5minutes with 25 ml of dilute hydrochloric acid, insoluble matter was collected in an ash less filter paper, washed with hot water and ignited to constant weight. The percentage of acid insoluble ash with reference to air dried drug was calculated.

b.3 DETERMINATION OF WATER SOLUBLE ASH

The water soluble ash obtained from total ash was boiled for 5 minutes with 25ml of distilled water. Insoluble matter was collected in an ash less filter paper, washed with hot water, and ignited for 15 minutes at a temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the ash; the difference in weigh represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug.

b.4 DETERMINATION OF SULPHATED ASH

Silica crucible was heated to redness for 10 minutes, allowed to cool in desiccators and weighed. 2g of the substance, accurately weighed, into the crucible, ignited gently at first, until the substance is thoroughly charred. Cool, moisten the residue with 1ml of sulphuric acid, heated gently until white fumes are no longer evolved and ignite at 800°C ± 25°C until all black particles have disappeared. The ignition was conducted in a place protected from air currents. The crucible was allowed to cool, add a few drops of sulphuric acid and heat. Ignite as before, cool and weighed. Operations were repeated until two successive weighing do not differ by more than 0.5 milligrams.

c. EXTRACTIVE VALUES

The extractive values obtained by exhausting the crude drug with solvent are indicative of the approximate measure of their chemical constituents. Taking into consideration the diversity in chemical nature and properties of contents of drugs, various solvents are used for determination of extractive values.

c.1 DETERMINAION OF ALCOHOL SOLUBLE EXRACTIVE VALUE

5g of the air dried coarsely powdered drug was macerated with 100ml of ethanol (95%) in a closed flask for twenty-four hours, shaking frequently (or in a mechanical shaker) during six hours and allowed to stand for eighteen hours. Rapidly

filtered, taking precautions against loss of solvent, evaporated 25ml of the filtrate to dryness in a tarred flat bottomed shallow dish and dried at 105°C to constant weight and weighed. The percentage of alcohol soluble extractive with reference to the air dried drug was calculated.

c.2 DETERMINATION OF WATER SOLUBLE EXRACTIVE VALUE

Weigh accurately about 5g of the coarsely powdered air dried drug and macerated with 1ml of chloroform water of the specified strength in a closed flask for twenty four hours, shaking frequently during six hours and allowed to stand for eighteen hours. Filtered rapidly, taking precautions against loss of solvent, evaporate 25ml of the filtrate to dryness in a tarred flat bottomed shallow dish and dried at 105°C to constant weight and weighed. The percentage of water soluble extractive was calculated with reference to the air dried drug.

c.3 DETERMINAION OF ETHER SOLUBLE EXTRACTIVE VALUE

5g of the substance accurately weighed and crushed drug to an extraction thimble, extracted with petroleum ether (boiling point 40°C to 60°C) in continuous extraction apparatus (Soxhlet extractor) for 6 hours. The extract was filtered into a tarred evaporating dish and solvent was evaporated on a water bath. The residue was dried at 105°C to constant weigh. The percentage of petroleum ether soluble extractive with reference to the air dried drug was calculated.

c.4 DETERMINATION OF HEXANE SOLUBLE EXRACTIVE

Weighed quantity about 5g of the air dried, crushed drug transferred to an extraction thimble and extracted with hexane in a continuous extraction apparatus (Soxhlet extractor) for 6 hours. Filtered the extract into tarred evaporating dish and evaporate the solvent on a water bath. The residue was dried at 105°C to constant

weight. The percentage of hexane soluble extractive was calculated with reference to the air dried drug.

c.5 DETERMINATION OF ETHYL ACETATE SOLUBLE

5g of accurately weighed quantity of the air dried, crushed drug was taken to an extraction thimble and extracted with ethyl acetate in a continuous extraction apparatus (soxhlet extractor) for 6 hours. The extract was filtered into a tarred evaporating dish and evaporated on a water bath. The residue was dried a 105°C to constant weight. The percentage of ethyl acetate soluble extractive was calculated with reference to the air dried drug.

d. DETERMINATION OF pH

The 5g of the sample was weighed and immersed in 100 ml of water in a beaker. The beaker was closed with aluminium foil and left behind for 24hours at room temperature. Later the supernatant solution was decanted into another beaker and the pH was determined using a calibrated pH meter.

6.2 PHYTOCHEMICAL ANALYSIS [12,16]

The plant material was subjected to preliminary phytochemical screening for the detection of various plant constituents.

- PREPARATION OF THE EXTRACT

The fine powdered drug was successfully extracted in an aspirated bottle with ethanol and then with water by cold maceration for 3-7 days. After than the supernatant is decanted and filtered with whatmann filter paper. The filtrate was boiled in a water bath to remove nearly 80 percent of the solvent and kept in vacuum oven and the residue obtained were used for further studies by preserving in refrigerator.

6.2.1 QUALITATIVE PHYTOCHEMICAL ANALYSIS

1. Triterpenoids (Salkowski test)

The extract was treated with few drops of concentrated sulphuric acid. Formation of yellow colour shows the presence of triterpenoids.

2. Flavones (Shinoda test)

To the extract in alcohol few magnesium turnings and few drops of concentrated hydrochloric acid were added and boiled for five minutes and red coloration was observed, which shows the presence of flavones.

3. Alkaloids (Dragendorff's reagent)

To the extract, added few drops of potassium bismuth iodide solution, reddish brown colour was observed, which indicates the presence of alkaloids.

4. Carbohydrates (Molisch's test)

In a test tube containing ethanolic (70 percent) extract of powdered drug, 2ml of distilled water and 2 drops of freshly prepared 20 percent alcoholic solution of α-naphthol was added. Mixed well and added 2ml of concentrated sulphuric acid along the sides of the test tube. Formation of red violet ring was observed, which disappears on addition of excess alkali solution, which confirms the presence of carbohydrates.

5. Glycosides (General test)

Extracted 200mg of drug treated with 5ml dilute sulphuric acid by warming on a water bath, filtered it and neutralized the acid extract with 5% solution of sodium hydroxide. Added 1ml of Fehling's solution A and B until it become alkaline (tested with pH paper) and heated on a water bath for 2minutes. Formation of red precipitate was observed, which indicates the presence of glycosides.

6. Phenols (Ferric chloride test)

Dissolve a small quantity of ethanolic extract (70 percent) of the drug with 2ml of distilled water, added a few drops 10% aqueous ferric chloride solution. A blue or green colour was produced, which indicates the presence of phenols.

7. Proteins (Biuret's test)

To 1ml of ethanolic extract of the drug, 5 to 8 drops of copper sulphate solution (10 percent) was added. Formation of violet colour indicates the presence of proteins.

8. Resin

Dissolve a small quantity of drug in ethanolic extract of the drug with 5-1 ml of acetic anhydride, heated gently. Cooled and added 0.05 ml of concentrated sulphuric acid. A bright purplish red colour was seen, which rapidly changed to violet. This indicates the presence of resins.

9. Saponins

To 5ml of an ethanolic extract of the drug, added a drop of sodium bicarbonate solution. Shake the mixture vigorously and left it for 3minutes. Honey comb like froth developed, which indicate the presence of saponins.

10. Tannins

The substance was mixed with basic lead acetate solution. Formation of white precipitate was observed, which indicates the presence of tannins.

11. Steroids (Liebermann- Burchard's test)

The extract was treated with few drops of acetic anhydride, boiled and cooled, and added concentrated sulphuric acid from the sides of the test tube. A brown ring was formed at the junction of two layers and upper layer turns green, which shows the presence of steroids.

6.3 FLUORESCENCE ANALYSIS [22,17]

Raw material used for the Isolation of solasodine and formulation of transdermal patches were studied for a colour changes in UV light. The samples were divided into three parts and each was studied under ordinary light, short UV (254 nm) and long UV (366 nm). Samples were studied as such, after treating with some chemicals.

6.4 QUALITATIVE AND QUANTITATIVE ESTIMATION OF HEAVY METALS AND INORGANIC ELEMENTS

Plant minerals play a vital role in metabolism. Presence of elements vary with the soil, climate conditions etc. There are essential and non-essential elements which may be beneficial or harmful to living things. Non-essential elements like lead, arsenic, cyanide, chromium, cadmium, aluminium, and silver bring about toxic effects resulting in intoxication. Hence, qualitative and quantitative estimation of inorganic elements in the plant *Solanum surattense* were carried out.

6.4.1 QUALITATIVE ANALYSIS OF INORGANIC ELEMENTS AND HEAVY METALS

To the ash of the drug material 50% v/v HCI was added and kept for 1 hour. It was filtered and the filtrate was used for the following tests.

1. Aluminium

   White gelatinous precipitate of aluminium hydroxide (Al (OH) $_3$) is formed on addition of ammonia solution. It is slightly soluble with excess of reagent. It dissolves in strong acid and base, but after boiling it is difficulty soluble.

2. Arsenic

   Arsenious salts in neutral solution react with solution of copper sulphate to form green precipitate (Scheele's green) which on boiling gives a red precipitate of cupric oxide.

3. Borate

   The mixture obtained by the addition of H_2SO_4 and alcohol (95%) to a borate. When ignited, burns with flame tinged with green.

4. Calcium

   Solution of calcium salts, when treated with ammonium carbonate solutions yield a white precipitate after boiling and cooling the mixture, is insoluble in ammonium chloride solution.

5. Carbonate

   Carbonate, when treated with dilute acid, effervescence ceases liberating CO2 which is colourless and produces a white precipitate in calcium hydroxide solution.

6. Chlorides

   Chlorides, when treated with silver nitrate solution yield a white curdy precipitate which is insoluble in nitric acid, but soluble after being well washed with water. In dilute ammonia solution, it is reprecipitated by the addition of nitric acid.

7. Copper

   An excess of ammonia, added to solution of cupric salt, which produces first a bluish precipitate and then a deep blue coloured solution.

8. Iron

   Solution of ferric salts, when treated with potassium ferro-cyanide solution, yields an intense blue precipitate which is insoluble in dilute HCI.

9. Lead

   Strong solution of lead salt, when treated with HCI, yield a white precipitate which is soluble in boiling water and re-deposited as crystals when the solution is cooled.

10. Magnesium

    Solution of magnesium salts, when treated with ammonium carbonate solution and boiled, yield a white precipitate, but yield no precipitate in the presence of ammonium chloride solution.

11. Mercury

    Solution of mercuric salts, when treated with sodium hydroxide solution, yields a yellow precipitate.

12. Nitrate

    With the solution of ferrous sulphate no brown colour was observed but if sulphuric acid is added (along the side of the test tube), a brown colour is produced at the junction of two liquids, indicating the presence of nitrates.

13. Phosphate

    Solution of phosphate when treated with silver nitrate solution yield yellow precipitate of normal silver orthophosphate Ag_3PO_4 (distinction from meta and para-phosphate) solution in dilute ammonia solution and in dilute nitric acid.

14. Potassium

    Moderately strong solution of potassium salts, which have been previously ignited to remove ammonium salts, when treated with perchloric acid (60%) yield a white crystalline precipitate.

15. Silver

    Solution of silver salts, when treated with potassium iodide solution yield a cream coloured precipitate which is insoluble in dilute ammonia solution and in nitric acid.

16. Sulpahte

    Solution of sulphate, when treated with lead acetate solution yields a white precipitate which is insoluble in ammonium acetate solution and in sodium hydroxide solution.

6.4.2 QUANTITATIVE ESTIMATION OF INORGANIC ELEMENTS

Instrumentation Parameters:

Instrument Name : Inductively Coupled Plasma Optical Emission Spectrometry.

Instrument Model : PE Optima 5300 DV ICP-OES;

Optical system Dual View – axial or radial

Detector system : Charge coupled detector, (UV – VISIBLE detector which is maintaining at -40^{0}C) to detect the intensity of the emission line wavelength range from 165 - 782 nm.

Torch (Light Source):

Positioned horizontally in the sample compartment along the central axis of the spectrometer optics. Changing from axial to radial viewing is a sample software command and is accomplished by computer control of a mirror located in the optical path. The torch assembly of this system comprises of two concentric quartz tubes.

Spray chamber : Scott type

Nebulizer : Common flow Gem tip

Preparation of samples by acid digestion method

Weighed 50mg of powdered mixture of powdered drug treated with acid mixture of sulphuric acid: water in the ratio of 4:1 in the Kjedahl flask and heated continuously till the solution is colourless. The sample mixture was then transferred in a 25ml volumetric flask and made up to the volume with distilled water.

Blank solution was prepared as above without sample.

The solution of Boron (B), Copper (Cu), Iron (Fe), zinc (Zn), Manganese (Mn) and Molybdenum (Mo) were prepared as per the protocol and the calibration curve was developed for each of them.

6.5 EXPERIMENTAL PLANT MATERIAL

6.5.1 EXTRACTION AND ISOLATION OF SOLASODINE [34]

The dried leaves and berries are first powdered and processed to remove fats using petroleum ether to give greenish yellow oil. This is rejected. The defatted material is extracted three times using ethanol. The combined extracts are concentrated to one tenth of its volume. Concentrated hydrochloric acid is added and the solution is refluxed for about six hours to allow complete hydrolysis of glycol alkaloid. The mixture is made alkaline by adding ammonia and refluxed for one hour. The reaction mixture is cooled and filtered and the residue obtained is thoroughly washed with water till the pH become neutral; is dried.

The dried material is dissolved in chloroform, where by solasodine mores into chloroform layer.

The solution is filtered and the solvent is evaporated to yield residue containing solasodine. It is further purified by crystallizing from methanol.

6.5.2 CHEMICAL PROFILE OF SOLASODINE

Drug name	:	Solasodine
Scientific name	:	(3β, 22α, 25R) – spirosol – 5 en-3-ol, solasod – 5-en-3βol, 20βF; 22αF, 25αF, 27-a2 aspiron -3β-01
Other name	:	solacarpidine, solanidine-s, pura purodine
Molecular formula	:	$C_{27}H_{43}NO_2$
Molecular weight	:	413.63 Da
Source	:	Steroid alkaloid isolated from various solanum species.
Crystalline structure	:	Hexagonal plate from MeoH

6.5.3 PHYSICAL PROPERTIES OF SOLASODINE

Melting point	:	198 – 200˚C
Optical rotation	:	$[\alpha]_{D25} = -98^{o}$, $[\alpha]_{D} = -98^{o}$ ($C^{0.14}$) in Me OH
PK_b	:	6.30
UV max (in Me OH)	:	206nm
Solubility	:	freely soluble in benzene, pyridine and chloroform.

6.5.4 CHARACTERIZATION OF ISOLATED COMPOUND [34,37]

TLC METHOD: Thin layer chromatography is a technique used for the separation, identification and estimation component. It is a reliable technique in which solute undergoes distribution between two phases, stationary and mobile phase. The separation is mainly based on the differential migration that occurs when a solvent flows along the thin layer of stationary phase. This may be achieved by partition and adsorption depending on stationary phase used.

1mg of the solasodine is dissolved in chloroform. 25µl of this solution was applied on Merck Aluminium plate pre coated with silica gel of 0.2mm thickness and the pate was developed using the mobile phase (10ml). The plate was dried and sprayed with Dragendroff's reagent. Plates are dried until it shows orange coloured spot.

TLC details:

Sample solution	: 1mg of solasodine in chloroform
Development system	: Toluene: Ethyl acetate: Diethlamine (7:2:1)
Stationary phase	: Silica gel 60 F254 TLC plate of 0.2mm thickness
Detection	: Drogendroff's reagent

6.5.5 HPTLC FINGER PRINTING AND QUANTIFICATION OF SOLASODINE [42]

HPTLC is High Performance Thin layer Chromatography or High Pressure Thin layer Chromatograph. This is a sophisticated advancement of Thin layer Chromatograph (TLC). HPTLC is one of the most versatile chromatographic methods. It has several advantages like better resolution, faster development of spots and also easy detection and quantification of separated compounds. The time required for the demonstration of most of the characteristic constituents of a sample or standards is very quick and short. In addition to qualitative detection, HPTLC also provides semi quantitative information. The fingerprint obtained is suitable for monitoring the identity and purity of drugs and for detecting adulterations and substitution in the sample.

CHROMATOGRAPHIC CONDITIONS

Table 1: Chromatographic conditions for HPTLC

INSTRUMENT	HPTLC
HPTLC APPLICATOR	CAMAG LINOMAT IV
HPLC SCANNER	CAMAG TLC SCANNER II
SAMPLE	SOLASODINE
MOBILE PHASE	Toluene: Ethyl acetate: Diethlamine (7:2:1)
STATIONARY PHASE	TLC SILICA GEL $60F_{254}$
SAMPLE DILUTION	Dissolved in 0.5ml chloroform
VOLUME APPLIED	20µl
Λ_{MAX}	206 nm

6.5.6 SPECTRAL ANALYSIS

The isolated solasodine was spectroscopically analysed for confirmation of its structure. Instrumental spectral analysis such as

IR SPECTROSCOPY

NMR – ^{1}H &^{13}C

MASS SPECTROSCOPY

IR SPECTROSCOPY [40,41]

IR spectrum is considered as vibrational-rotational spectra. KBr pellet technique was used for solid compound, for liquid compound Nujol mull method was followed. This method is much helpful which would give information about functional group present in the organic compounds. Mechanism of bond stretching and bending is happened when electromagnetic radiation ranging from 500cm^{-1} to 4000 cm^{-1} passed through the sample.

NMR SPECTROSCOPY [41]

A nuclear magnetic spectrum is used o detect chemical structure of the molecules. When sample absorbs radiation at different radio frequency region, it will cause the excitation of proton or nuclei present in the sample against magnetic field. The solvent used for dissolving the sample was deuterated chloroform.

^{1}H NMR

Most commonly used NMR is proton NMR, because of the sensitivity and wide range of characteristic information. Range of chemical shift (δ) is from 0-14ppm. Chemical shift of the test compound was compared with TMS protons which are attributed at 0ppm.

^{13}C NMR

It is new technique but natural abundance is very low of about 1.1%. So, this further reduces the sensitivity of absorption. The range of chemical shift (δ) is from 0-180ppm. TMS is used as internal reference. The functional group containing carbon atom can be determined.

MASS SPECTROSCOPY [39,34]

It is an accurate method to determine the molecular mass of the compound. The spectrum tells about the mass, relative abundance of the molecular ions and positively charged fragments formed by electronic bombardment. Sample was dissolved in $CDCl_3$ and injected through direct probe inlet. Electron impact ionization method was used.

6.6 ACUTE ORAL TOXICITY

The acute oral toxicity study was carried out as per the guidelines set by organization for economic co-operation and development (OECD), received draft guidelines 423, received from committee for the purpose of control and supervision of experiments on animals (CPCSEA), ministry of social justice and empowerment, government of India. Swiss albino mice of either sex weighed between 18 – 22g wee fasted overnight prior to the acute experimental procedure.

The principle, is based on a stepwise procedure with the use of minimum number of animals per step. The LD 50 of isolated compound solasodine was determined. The therapeutic dose was calculated as $1/10^{th}$ of the lethal dose for further investigation.

6.7 FORMULATION DEVELOPMENT [8]

Basic components of transdermal patches

1. POLYMER MATRIX:

The polymer controls the release of drug from the device. The following criteria should be satisfied for a polymer to be used in a transdermal system.

i) Molecular weight, glass transition temperature and chemical functionality of the polymer should be such that the specific drug diffuses properly and gets released through it.

ii) The polymer should be stable, non-reactive with the drug, easily manufactured and fabricated into the desired product; and inexpensive.

iii) The polymer and its degradation product must be nontoxic or non-antagonistic to the most.

iv) The mechanical properties of the polymer should not deteriorate excessively when large amount of active agent are incorporated into it.

Possible useful polymers for transdermal devices are,

Natural polymers:

Cellulose derivatives, zein, gelatin, shellac, waxes, proteins, gums and their derivatives, natural rubber, starch etc.

Synthetic elastomers:

Poly butadiene, poly siloxane, silicone rubber, aceryl nitrile etc.

Synthetic polymers:

PVA & PVP, polyvinyl chloride, polyethylene, polypropylene etc.

2. DRUG:

The following are some of the desirable properties of a drug for transdermal delivery.

Physiochemical Properties:

i) The drug should have a molecular weigh less than approximately 1000 daltons.
ii) The drug should have affinity for both lipophilic and hydrophilic phases.
iii) The drug should have a less melting point.

Biological Properties:

i) The drug should be potent with a daily dose of the order of a few mg/day.
ii) The half life ($t_{1/2}$) of the drug should be short.
iii) The drug must not induce a cutaneous irritant or allergic response.
iv) The drugs which have to be administered for a long period of time or which cause adverse effects to non-target issues can also be formulated for transdermal delivery.

3. PERMEATION ENHANCERS:

A large number of compounds have been investigated for their ability to enhance stratum corneum permeability. They are conveniently classified as follows.

i) Solvents: methanol, ethanol, dimethyl sulfoxide, dimethyl acetamide, dimethyl formamide, propylene glycol, glycerol, isopropyl palmitate etc.
ii) Surfactants: anionic surfactants – sodium lauryl sulphate, decodecyl methyl sulphoxide etc.
Non Ionics surfactants – plutonic F127, pluronic F68 etc.
Bile salts – sodium taurocholate, sodium deoxycholate, sodium tauroglycocholate.

4. OTHER EXCIPIENTS:

   Adhesives: some widely used pressure sensitive adhesives include poly isobuthylenes, acrylics and silicones.

5. BACKING MEMBRANE:

   It is impermeable substance that protects the product during use on the skin eg: metallic plastic laminate, plastic backing with absorbent pad and occlusive base plate (aluminium foil), adhesive foam pad (flexible poly urethane) with occlusive base plate (aluminium foil disc) etc.

6.7.1 METHODS FOR PREPARATION OF PATCHES [45,51]

METHOD 1: Ethyl cellulose, PVP was used as the skeletal material of preparation. propylene glycol as penetration enhancer. PVP (1g) and ethyl cellulose (1g) were weighed in requisite ratios and mixed in 10ml distilled water, stirred the mixture over a hot water bath until dissolved. After the mixture was cooled down to 25^{o}c, solasodine 500mg (5g), propylene glycol (0.5ml), glycerol (0.5ml) were added. The mixture was then poured into glass moulds and dried at room temperature for 24 hrs. The patches were removed by peeling and cut into required size.

METHOD 2: Transdermal patches were prepared by solvent evaporation technique. The polymer (HPMC) and isolated compound of solasodine were weighed. PEG ,which acts as plasticizer and permeation enhancer, was used in the concentration of 30% v/v. ethanol was used as a solvent. PEG 2.68ml (30% weigh of polymer) was dissolved in ethanol with stirring. The calculated amount of HPMC (1000mg) was dispersed in solvent ethanol. Isolated Solasodine 500mg was dissolved in ethanol; this solution was then added to polymer base and stirred continuously to get uniform solution. This solution was poured into Petri plate coated with liquid paraffin and then dried a room temperature. After during, patches were removed and cut into required sizes and used for further studies

FORMULA FOR PREPARATION:

Table 2: Formula for the preparation of Transdermal patches

CODE INGREDIANTS	FORMULATION 1	FORMULATION 2
Ehyl cellulose	1000mg	-
PVP	1000mg	-
HPMC	-	1000mg
Propylene glycol	0.5ml	2.68ml
Solvent	500ml	500ml
Solasodine	500mg	500mg

6.7.2 EVALUATION OF MEDICATED TRANSDERMAL PATCHES [23,50]

1) WEIGHT VARIATION TEST:

The study was carried out on 9 films. The mean weight of the film as well as deviation from the mean was obtained.

2) DETERMINATION OF FOLDING ENDURANCE:

A patch was repeatedly folded at the same place till it broke. The number of times the film could be folded at the same place without breaking gave the value of the folding endurance.

3) PERCENTAGE MOISTURE CONTENT:

The prepared films were weighed individually and kept in a desicators containing fused calcium chloride at RT for 24 hrs. After 24hrs the films were reweighed and the percentage moisture content was calculated by the given formula,

$$\text{Percentage moisture content} = \frac{\text{initial weight - final weight}}{\text{Initial weight}} \times 100$$

4) DRUG CONTENT STUDY:

Transdermal patches were taken (2 cm2 areas) individually, crushed and taken in a 100ml volumetric flask (pH 7.4 phosphate buffer). The medicine was stirred with Teflon – coated magnetic bed for 24hrs. The contents were filtered using wahtmann filter paper and the suitable phosphate buffer pH 7.4. Absorbance of dilutions was measured by using UV – VIS spectrometer at 206nm against phosphate buffer pH 7.4 as a blank.

6.7.2 *IN VITRO* EVALUATION STUDY OF TRANSDERMAL PATCHES [50]

The *in vitro* permeation experiments were conducted using Franz diffusion cell (Receptor compartment capacity: 20ml). Cellophane membrane is used. The receiver compartment was filled with 20ml of 10% hydroalcoholic phosphate buffer, pH7.4. The transdermal patch was firmly pressed into the center of the membrane and then the cellophane membrane is mounted to the donor compartment. The donor compartment was then placed in position such that the surface of the membrane touches the receptor fluid surface. The whole assembly was kept on a thermostatically controlled magnetic stirrer set at 37 ± 2^{o}c and the content in the receiver compartment was continuously stirred at a constant speed (100 rpm) using a magnetic bead. The samples (2ml) were withdrawn at the intervals of half an hour up to 6hrs and replaced with same amount (2ml) of 10% hydro alcoholic phosphate buffer to maintain the membrane condition.

The samples were analyzed for drug content using UV – VIS spectrophotometer at 206nm. The cumulative % drug release from the transdermal patch of solasodine was calculated.

6.8 *INVIVO* EVALUATION OF TRANSDERMAL PATCHES

Animals were procured on the approval given by Animal ethical committee, Madras Medical College, Chennai-03. Clearence No: Vide.3/243/CPCSEA.

Skin Irritation Study

Healthy male albino rabbits weighed 1.5 – 2.5 kg are divided into 2 groups containing 3 animals each. On the test day the dorsal surface of each rabbit is shaved prior to the experiment. Group I (control) treated with the transdermal patches without drug. Group II (test) treated with transdermal patches containing test drug. The patches are applied to intact skin for hours. The patches are then removed after hours of exposure period and the formation of any erythema or edema is observed at 24, 48 and 72 hours. The observation was made for 14 days to determine any persistent or delayed effects.

6.9 *INVIVO* EVALUATION FOR ANTI INFLAMMATORY ANTI PYRETIC AND ANALGESIC ACTIVITY [55,56,57,58]

6.9.1 ANTI INFLAMMATORY STUDIES (CARRAGEENAN INDUCED PAW OEDEMA IN RATS) [54]

The paw oedema was induced by 0.1ml of 1% (w/v) carrageenan suspension into the sub-planter region of right hind paw of rats. The control group (A) was orally administered saline (10ml/kg) while the standard group (B) was given indomethacin (5mg/kg) and group (C) patches were applied topically one hour before carrageenan injection. The inhibitory activity was calculated according to the formula,

$$\% \text{ inhibition} = \frac{(Ct-Co)conrol-(Ct-Co)treated)}{(Ct-C0)control} \times 100$$

Where, C_t is the paw circumference at time t, C_o is the paw circumference before carrageenan injection and ($C_t - C_o$) is oedema or change in paw size after time t.

6.9.2 EVALUATION OF ANTIPYRETIC ACTIVITY

The antipyretic efficacy of solasodine TDS was assessed using brewer's yeast induced pyrexia method. Pyrexia was induced by injecting 10 ml/kg of 20% w/v suspension of Brewer's yeast in normal saline subcutaneously 18 hours before the commencement of experiment. Only those animals, whose rectal temperature is increased by at least 1^{o}c after 18 hours of induced subcutaneous yeast injection, were included in the study. The normal body temperature of each animal was measured using digital thermometer inserting into rectum. The experimental animals randomly selected were divided into 3 groups. The control group (A) was orally administered saline (10 ml/kg) while the standard group (B) was given (100 mg/kg) aspirin and group (C) treated transdermal patches. The rectal temperature was recorded at time interval of 1 hour, 2 hr, 3 hr, 4 hr and 5 hrs after drug administration.

6.9.3 EVALUATION OF ANALGESIC ACTIVITY

The peripheral analgesic activity of test drug was evaluated in acetic acid induced writing experiment using mice. The abdominal constriction writings resulting from intraperitoneal injection of acetic acid (10 ml/kg of 0.6% v/v glacial acetic acid solution in water) were observed according to standard procedure. 10 ml/kg saline was orally administered to group A (control group) whereas standard aspirin (100 mg/kg) was prescribed for group (B) and transdermal patches are applied for group (C). Acetic acid solution was administered after 30 min and number of writings counted in each animal for 15 min. Percentage inhibition response was calculated as the reduction in the number of abdominal constrictions between control and test treated groups as a percentage number of witness observed in case of control group.

7. RESULTS AND DISCUSSION

7.1 ANALYSIS OF RAW MATERIALS

7.1.1 PRELIMINARY QUALITY CONTROL OF RAW MATERIALS

The raw materials were sampled, authenticated and studied for their compliance to primary

Qualitative standards as established by Ayurvedic pharmacopoeia of India and other standard reference books. For those, whose standard values were unavailable, in-house standards were fixed based on the results obtained from the preliminary test of the raw material.

Authentication of *Solanum surattense*:

The plant was authenticated by Prof.P.Jayaraman, Botanist, Tambaram, Chennai.

7.1.2 PHYSIOCHEMICAL EVALUATION

A) MOISTURE CONTENT / LOSS IN DRYING:

The results obtained and the standard values are shown in below table.

Table 3: Loss on drying

Ingredient	Loss on drying (% w/w)	Standard (% w/w)
Leaves of *S.surattense*	3.4 ±0.55	NMT 12%
Berries of *S.surattense*	3.2 ±0.5	

Note: The test is preferred in triplicate and results are given as mean ± standard deviation;

NMT – not more than.

From the table it is observed that *Solanum surattense* low moisture content.

B.1) TOTAL ASH:

The values obtained and their acceptable limits defined are given in table 4.

Table 4: Total ash value

Ingredient	Total ash (% w/w)	Standard (% w/w)
Leaves of *S.surattense*	6.2 ±0.76	NMT 9%
Berries of *S.surattense*	5.9 ±0.50	

Note: The test is preferred in triplicate and results are given as mean ± standard deviation;

NMT – not more than.

The total ash content of *Solanum surattense* complies with the standard value.

B.2) ACID INSOLUBLE ASH:

From the total ash content, the acid insoluble ash of solanum surattense was determined and results are enumerated in table 5.

Table 5 : Acid insoluble ash

Ingredient	Acid insoluble ash (% w/w)	Standard (% w/w)
Leaves of *S.surattense*	1.04 ±0.13	NMT 3%
Berries of *S.surattense*	1.45 ±0.55	

Note: The tests are preferred in triplicate and results are given as mean ± standard deviation;

NMT – not more than.

Acid insoluble ash value of *Solanum surattense* complies with the standard value.

B.3) WATER SOLUBLE ASH:

The water soluble ash was determined for the crude drug and results are enumerated in table 6.

Table 6: Water soluble ash

Ingredient	Water soluble ash (% w/w)	Standard (% w/w)
Leaves of *S.surattense*	1.65 ±0.58	NMT 2%
Berries of *S.surattense*	1.90 ±0.71	

Note: The tests are preferred in triplicate and results are given as mean ± standard deviation;

NMT – not more than.

Water soluble ash values of the crude drug, complies with the standard value.

B.4) SULPHATED ASH:

The sulphated ash value for the crude drug was listed in table 7.

Table 7: Sulphated ash value

Ingredient	Sulphated ash (% w/w)	Standard arrived (% w/w)
Leaves of *S.surattense*	3.40 ±0.56	NMT 4%
Berries of *S.surattense*	1.99 ±0.56	

Note: The tests are preferred in triplicate and results are given as mean ± standard deviation;

NMT – not more than.

The sulphated ash value for the crude drug complies with the standard value.

C.1) WATER SOLUBLE EXTRACTIVE VALUES:

The extractive values for the raw materials were determined in water and the results are tabulated in table 8.

Table 8: Water soluble extractive value

Ingredient	Water soluble extractive (% w/w)	Standard arrived (% w/w)
Leaves of *S.surattense*	21.3 ±1.56	NLT 16%
Berries of *S.surattense*	18.9 ±0.72	

Note: The tests are preferred in triplicate and results are given as mean ± standard deviation;

NLT – not less than.

C.2) ALCOHOL SOLUBLE EXTRACTIVE:

The extractive values were determined in alcohol (90% ethanol) and the results are tabulated in table 9.

Table 9: Alcohol soluble extractive value

Ingredient	Alcohol soluble extractive (% w/w)	Standard arrived (% w/w)
Leaves of *S.surattense*	10.7 ±0.81	NLT 6%
Berries of *S.surattense*	12.6 ±0.50	

Note: The tests are preferred in triplicate and results are given as mean ± standard deviation;

NLT – not less than.

C.3) HEXANE SOLUBLE EXTRACTIVE:

The extractive values were determined in hexane and the results are tabulated in table 10.

Table 10: Hexane soluble extractive

Ingredient	Hexane soluble extractive (% w/w)
Leaves of *S.surattense*	20.6 ±1.45
Berries of *S.surattense*	20.2 ±0.83

Note: The tests are preferred in triplicate and results are given as mean ± standard deviation;

NLT – not less than.

C.4) ETHYL ACETATE SOLUBLE EXTRACTIVE VALUES:

The extractive values were determined using ethyl acetate and the results are tabulated in table 11.

Table 11: Ethyl acetate soluble extractive value

Ingredients	Ethyl acetate soluble extractive (% w/w)
Leaves of *S.surattense*	20.8 ±2.35
Berries of *S.surattense*	17.4 ±1.05

Note: The tests are preferred in triplicate and results are given as mean ± standard deviation;

NLT – not less than.

7.2 PHYTOCHEMICAL ANALYSIS

Qualitative phytochemical analysis:

The plant material was subjected to preliminary phytochemical analysis and the results are tabulated in the below table 12.

Table 12:

	Phytoconstituents	Leaves of *S.surretense*	Berries of *S.surretense*
1)	Triterpenoids	-	-
2)	Flavones	+	+
3)	Alkaloids	+	+
4)	Carbohydrates	+	+
5)	Glycosides	+	+
6)	Phenols	+	+
7)	Proteins	+	+
8)	Resin	-	-
9)	Saponins	+	+
10)	Tannins	-	-
11)	Steroids	+	+

7.3 FLOURESCENCE ANALYSIS

The raw materials used for the isolation of solasodine and formulation of TDS were subjected to fluorescence analysis under day light and UV light. The results are tabulated in table 13.

Table 13: Flourescence analysis for the leaves of *Solanum surattense*

Powdered drug	Day Light	UV Light	
		Short	Long
1) Powder + H_2O	Light brown	Dark brown	Green
2) Powder	Green	Brown	Green
3) Powder + 1N Hcl	Green	Dark Brown	Green
4) Powder + 1N H_2SO_4	Dark brown	Dark green	Brown
5) Powder + 1N HNO_3	Greenish black	Dark green	Dark Green
6) Powder + CH_3COOH	Light green	Greenish black	Dark Brown
7) Powder + NaoH	Pale green	Brown	Dark Orange
8) Powder + Alc. NaoH	Light green	Dark green	Green
9) Powder + 1N KOH	Light green	Greenish yellow	Green
10) Powder + Alc.KOH	Greenish brown	Dark green	Brown
11) Powder + NH_3	Pale green	Yellowish green	Yellow
12) Powder + I_2	Greenish orange	Pale orange	Light Green
13) Powder + $FeCl_3$	Brownish green	Reddish brown	Brown
14) Powder + ethanol	Dark green	Pale green	Green

Table 14: Flourescence analysis for the berries of *Solanum surattense*

Powdered drug	Day Light	UV Light	
		Short	Long
1) Powder + H_2O	Brown	Brown	Dark brown
2) Powder	Pale Green	Brown	Green
3) Powder + 1N Hcl	Greenish yellow	Dark Brown	Yellow
4) Powder + 1N H_2SO_4	Dark brown	Dark green	Brown
5) Powder + 1N HNO_3	Greenish black	Dark green	Dark Green
6) Powder + CH_3COOH	Light green	Dark brown	Brown
7) Powder + NaoH	Pale green	Brown	Dark Orange
8) Powder + Alc. NaoH	Light green	Dark green	Green
9) Powder + 1N KOH	Green	Greenish yellow	Green
10) Powder + Alc.KOH	Greenish brown	Dark green	Brown
11) Powder + NH_3	Pale green	Yellow	Yellow
12) Powder + I_2	Greenish orange	Pale orange	Orange
13) Powder + $FeCl_3$	Brown	Brown black	Dark green
14) Powder + ethanol	Dark green	Pale green	Green

7.4 INORGANIC ELEMENTS AND HEAVY METAL ANALYSIS

Qualitative and quantitative estimation of inorganic metals was analysed using inductively coupled plasma optical emission spectrometry and the results were tabulated.

Table 15: Qualitative estimation of inorganic elements and heavy metals

S.NO	INORGANIC ELEMENTS	Leaves of *S.surretense*	Berries of *S.surretense*
1	Aluminium	+	+
2	Arsenic	+	+
3	Boron	+	+
4	Calcium	+	-
5	Carbon	-	-
6	Chloride	-	+
7	Copper	+	+
8	Iron	+	+
9	Lead	+	+
10	Magnesium	-	-
11	Mercury	-	-
12	Nitrate	-	-
13	Phosphate	-	-
14	Potassium	+	+
15	Silver	-	-
16	Sulfur	+	+
17	Sodium	+	+
18	Zinc	+	+
19	Manganese	+	+
20	Molybdenum	+	+

7.4.1 QUANTATIVE ESTIMATION OF INORGANIC ELEMENTS OF *Solanum surattense*

Table 16: Quantitative estimation of Inorganic elements.

INORGANIC ELEMENS	TOTAL AMOUNT IN LEAVES (%w/w)	TOTAL AMOUNT IN BERRIES (%w/w)
Calcium	0.82	-
Potassium	2.68	1.92
Sodium	1.72	1.88
Aluminium	1.30	1.31
Sulfur	0.44	1.09

7.4.2 HEAVY MEAL ANALYSIS OF *Solanum suratense*

Table 17: Heavy metal analysis

HEAVY METALS	TOTAL AMOUNT IN LEAVES	TOTAL AMOUNT IN BERRIES	STANDARD VALUE
Mercury	0.0325 ppm	0.0367	NMT 0.5 ppm
Arsenic	0.9840 ppm	0.7680	NMT 5.0 ppm
Cadmium	0.0257 ppm	0.1589	NMT 0.3 ppm
Iron	1.0435 ppm	0.9887	NMT 10 ppm
Lead	3.9080 ppm	2.5801	NMT 10 ppm

7.5 CHARACTERISATION OF ISOLATED SOLASODINE

7.5.1 TLC

Solvent system : Toulene: ethyl acetate: diethyl amine (7:2:1)

Detecting agent : Dragendroff's reagent

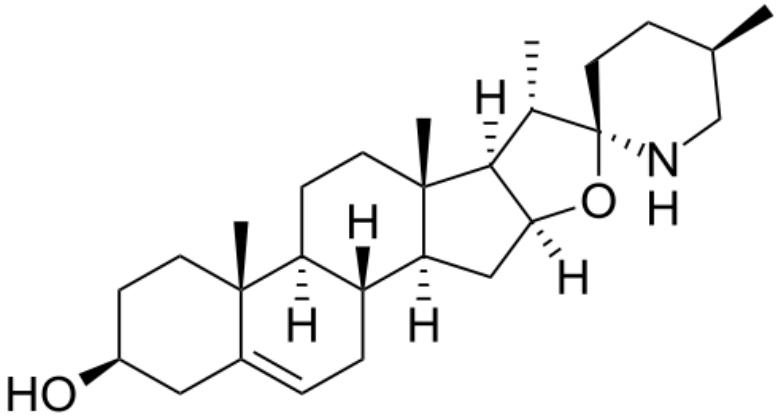
Colour of the spot: Orange

R_F value : $\frac{Distance\ moved\ by\ solute}{Distance\ moved\ by\ solvent}$

R_F value of the isolated compound of solasodine = 3.7 / 5.9 cm

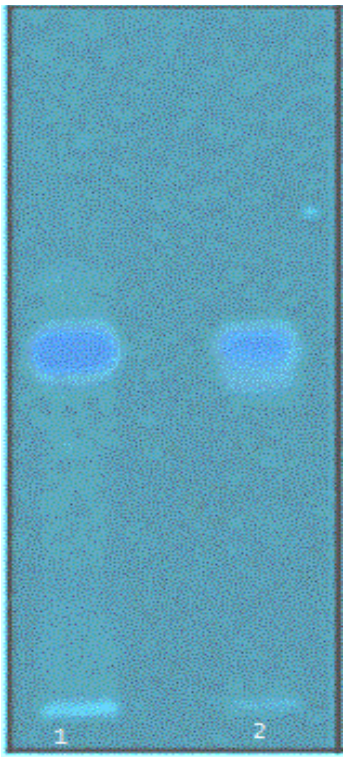
= 0.62

Standard value of solasodine = 0.6



7.5.2 HPTLC OF SOLASODINE

Fig 13: HPTLC Plate



QUANTIFICATION OF SOLASODINE BY HPTLC

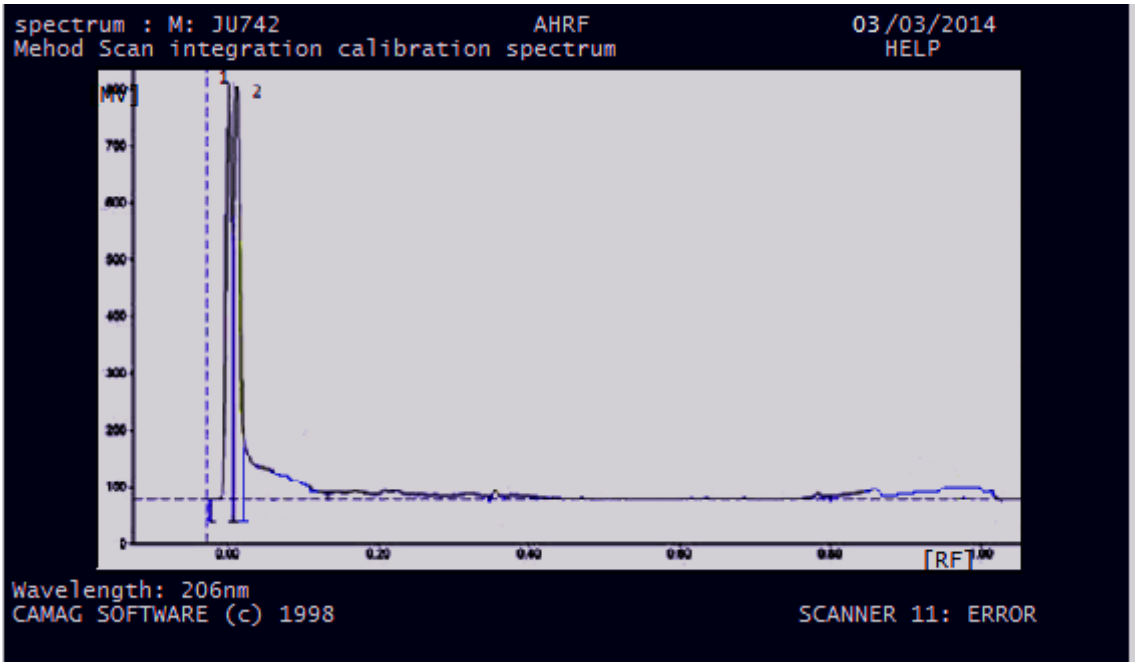


Fig 14: HPTLC of Solasodine

Table 18: HPTLC of Solasodine

Sample	R_f	Retention time (min)	Area	Solasodine content (mg/g)	Total solasodine content (mg)	solasodine (%)
S1	0.6	15.5	1211648	9.93	227.7	99.9%
S2	0.62	15.1	171590	7.8	213.0	86.3%

7.5.3 SPECTRAL ANALSIS OF ISOLATED COMPOUND SOLASDINE

7.5.3.1 IR SPECTRUM OF ISOLATED SOLASODINE

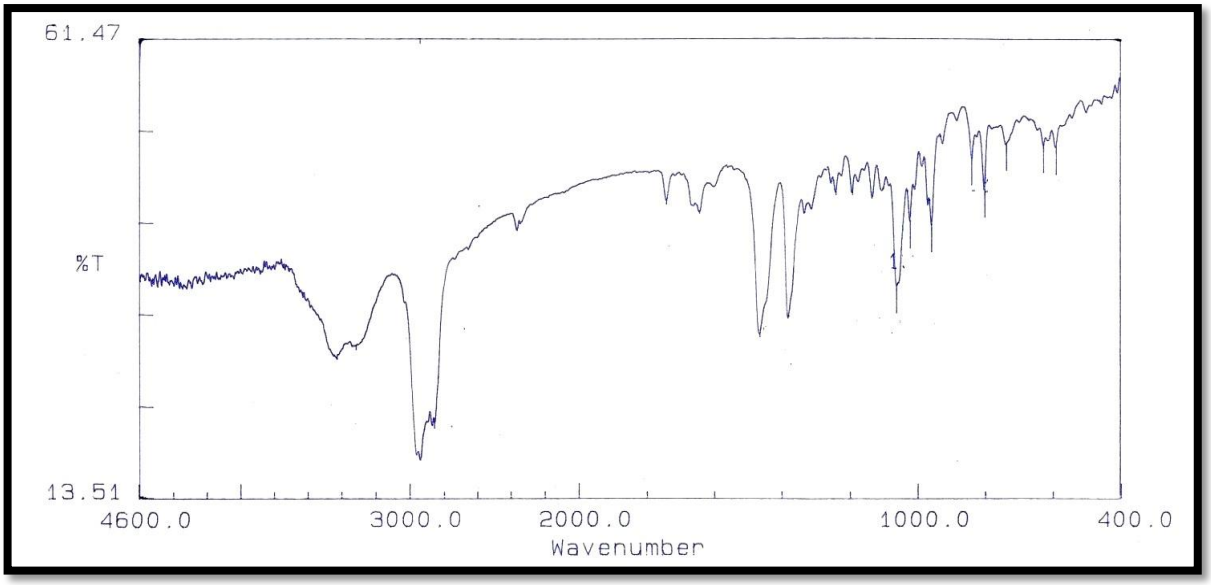


Fig 15: IR spectrum of solasodine

IR: Kbr pellet technique

Table 19:

FUNCTIONAL GROUP	AUTHENTICATED SAMPLE ν cm^{-1}	ISOLATED COMPOUND ν cm^{-1}
C-O	1070.6	1066.4
C-N	1136.5	1182.8
C-H	1452.9 2940.5	1464.8 2945.4
C=C	1675.2	1690.1
N-H	1640.8 3258.9	1589.3 3241.9
OH	3448.9	3456.4

7.5.3.2 ^{13}C NMR SPECTROSCOPY OF ISOLATED SOLASODINE

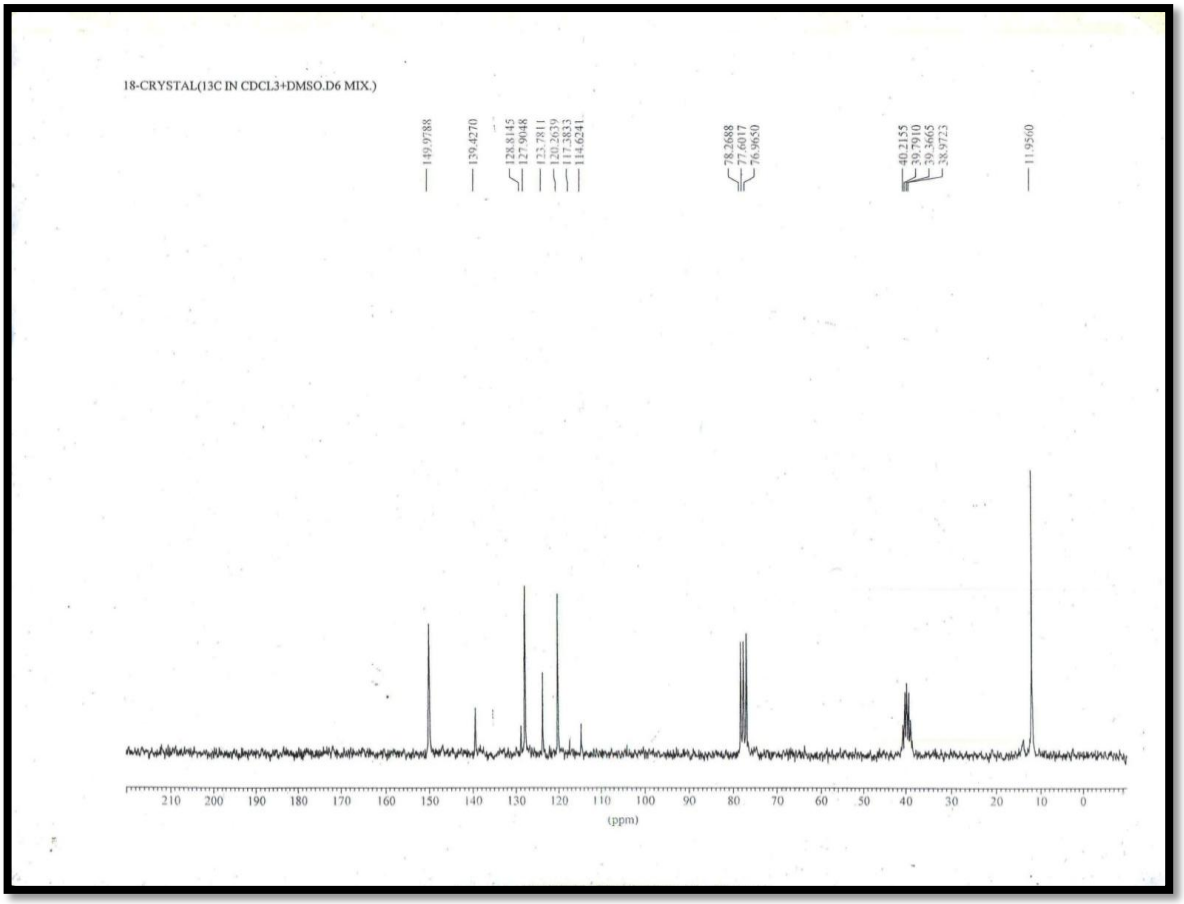


Fig 16: ^{13}C NMR of solasodine

^{13}C-NMR (CD3OD, 125.76 MHz): δc 37.1 (C-1), 30.9 (C-2), 70.8 (C-3), 41.6 (C-4), 140.9 (C-5), 120.8 (C-6), 31.6 (C-7), 31.8 (C-8), 50.3(C-9), 36.7 (C-10), 20.6 (C-11), 39.6 (C- 12), 40.5 (C-13), 56.3 (C-14), 32.0 (C-15), 78.8 (C-16), 62.7 (C-17), 19.3 (C-18), 16.3 (C-19), 41.3 (C-20), 15.2 (C-21), 98.1 (C-22), 33.5 (C-23), 29.3 (C-24),30.2 (C- 25), 47.3(C-26), 19.8 (C-27).

^{1}H NMR OF ISOLATED SOLASODINE

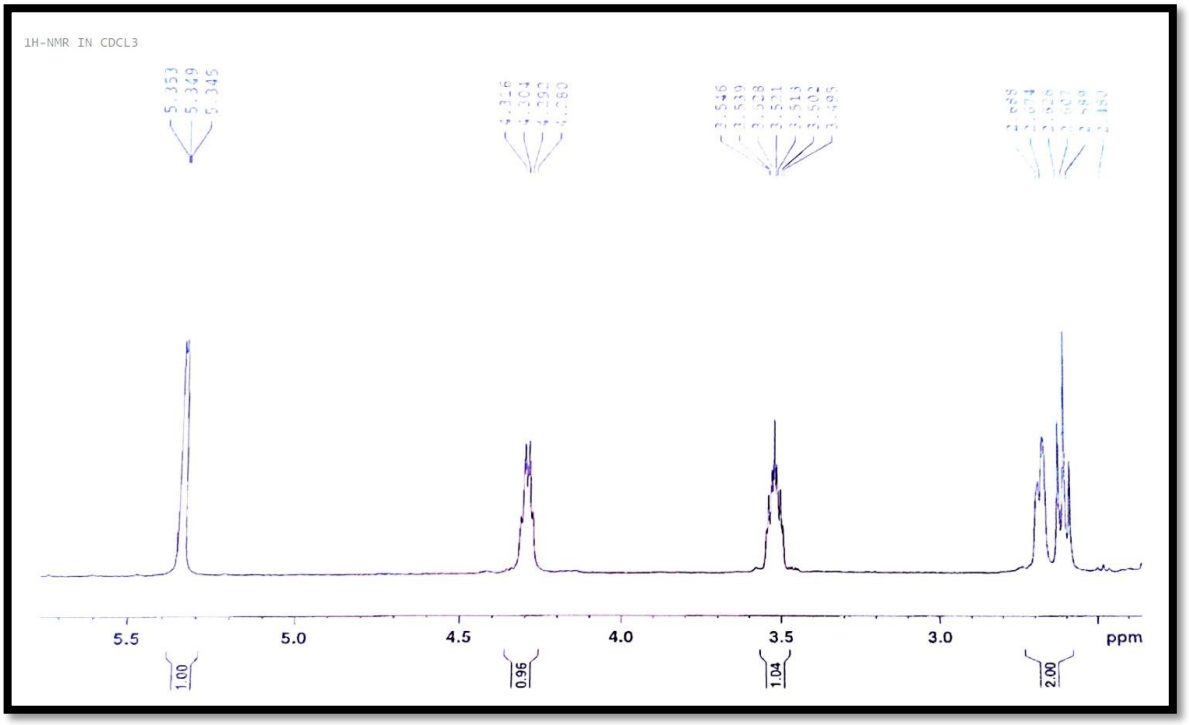


Fig 17: ^{1}H NMR of solasodine

Table 20: 1H-NMR: (CD3OD, 500.13 MHz)

δ VALUE	FUNCTIONAL GROUP
7.26	Benzene ring
1.45	-CH_3
1.25	-CH_2
0.94	-CH_3
2	-OH gr
5.2	-NH

7.5.3.3 MASS SPECTRUM OF ISOLATED SOLASODINE

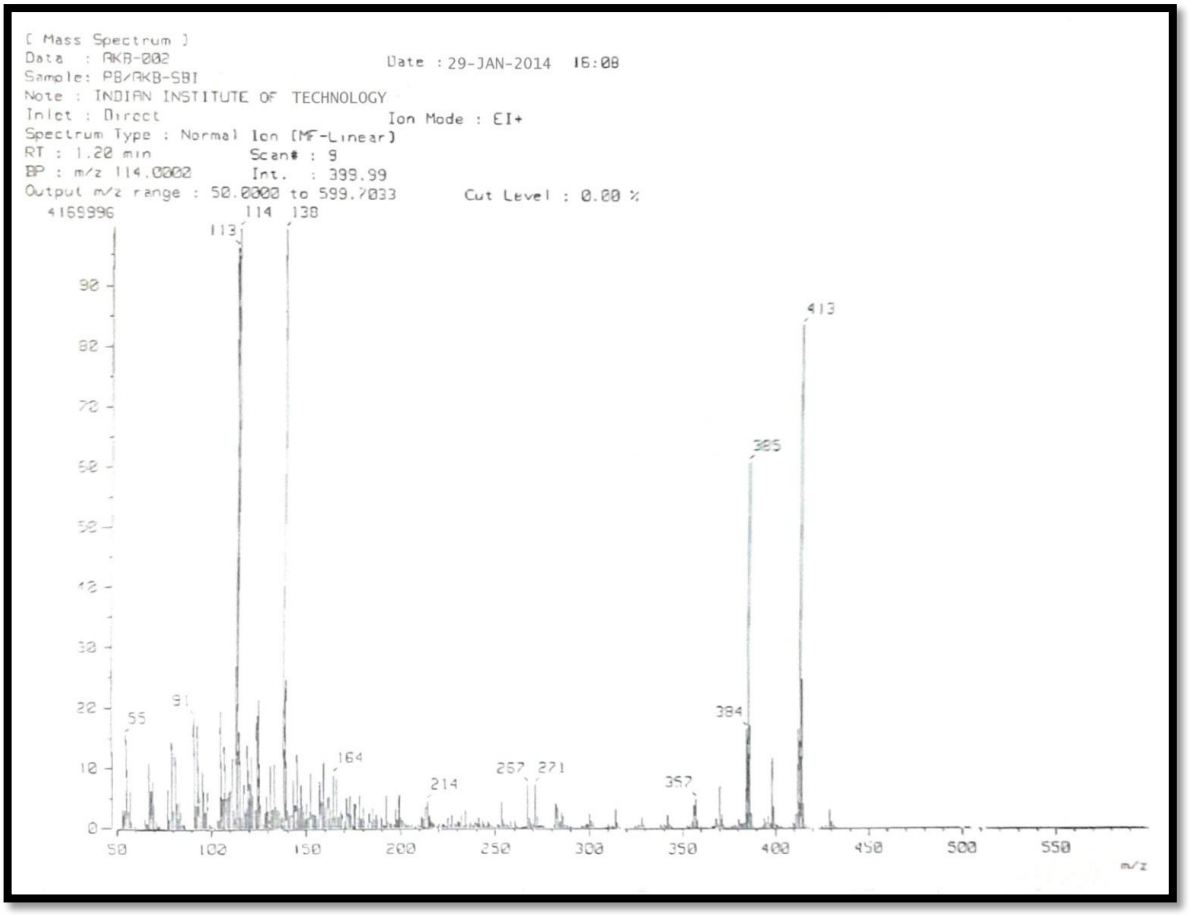


Fig 18: MASS spectrum of solasodine

M^+ ion peak (Molecular ion peak) : 412

Base peak : 130

7.6 EVALUATION OF MEDICATED TRANSDERMAL PATCHES

1. UNIFORMITY OF WEIGHT

The weight uniformity of Transdermal patches was evaluated and the results are shown in (Table:21). The weight of the films was observed between 0.0159 to 0.0160g from the results of mean and standard deviation. All the patches were found to contain an almost uniform weight.

2. DRUG CONTENT

The drug content was studied by using UV- Spectrophotometer at 206nm. All the patches were found to contain 92.9 to 98.7% w/w of drug . (Table:22)

3. MOISTURE ABSORPTION STUDIES:

The results of percentage moisture absorption studies are shown in (Table:23). The percentage moisture absorbed range between 2.4155 to 2.4590 %w/w.

4. PERCENTAGE MOISTURE LOSS

The results of percentage moisture loss studies are shown in (Table:24). The percentage moisture loss ranges between 1.201 to 1.207 %w/w. From the results indicate that all the patches were stable in dry condition and retain its integrity.

5. SURFACE pH

The pH of the film was found by using pH paper. The results are shown in (Table:25). All the patches have around neutral pH 6. So it will not cause any kind of irritation to the skin.

6. FOLDING ENDURANCE

Folding endurance was measured manually and the results are shown in (Table:26). All the patches scored good folding endurance (>200), which are considered satisfactory.

1.UNIFORMITY IN WEIGHT

Table 21:

CODE	FORMULATION 1	FORMULATION 2
POLYMERS	E.C + PVP	HPMC
WEIGHT in g	0.0159	0.0161
	0.0158	0.0158
	0.0162	0.0157
MEAN ± SD	0.0159 ±0.0014	0.0159 ±0.0017

2. PERCENTAGE DRUG CONTENT

Table 22:

CODE	FORMULATION 1	FORMULATION 2
POLYMER	E.C + PVP	HPMC
% DRUG CONTENT	92.912	98.758
	93.019	98.448
	92.858	98.811
MEAN ±SD	92.929± 0.0211	98.775± 0.0249

3. PERCENTAGE MOISTURE ABSORBANCE

Table 23:

CODE	FORMULATION 1	FORMULATION 2
POLYMER	EC + PVP	HPMC
% MOISTURE ABSORBANCE	2.4162	2.4540
	2.4155	2.4691
	2.4150	2.4539
MEAN±SD	2.4155 ±0.002	2.459 ±0.001

4. PERCENTAGE MOISTURE LOSS

Table 24:

CODE	FORMULAION 1	FORMULATION 2
POLYMER	E.C + PVP	HPMC
% MOISTURE LOSS	1.205	1.198
	1.199	1.197
	1.200	0.988
MEAN±SD	1.201 ±0.004	1.127 ±0.17

5. SURFACE pH

Table 25:

CODE	FORMULATION 1	FORMULATION 2
POLYMER	E.C + PVP	HPMC
pH	6	6

6. FOLDING ENDURANCE

Table 26:

CODE	FORMULATION 1	FORMULATION 2
POLYMER	E.C + PVP	HPMC
FOLDING ENDURENCE	>200	>200

7.7 *IN VITRO* EVALUATION OF TDS

FORMULATION 1

Table 27: *In vitro* evaluation of Formulation-1

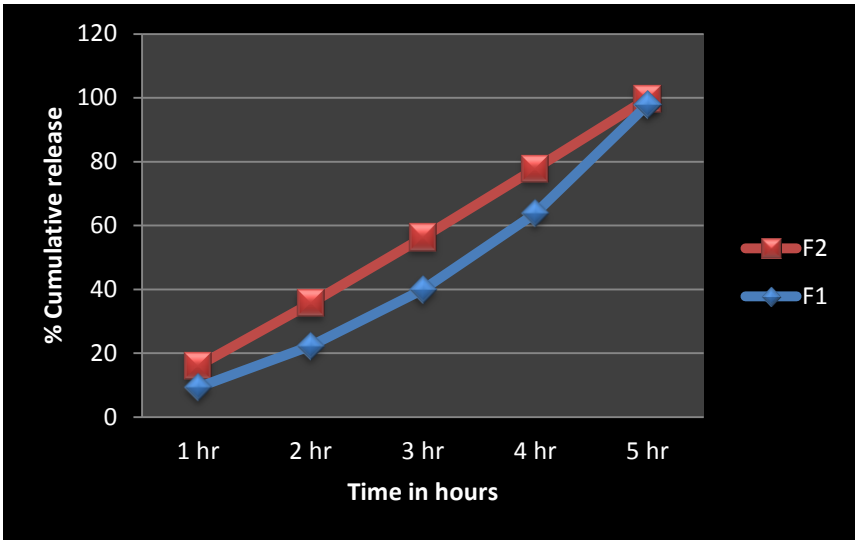
	Abs (nm)	Con. In µg/ml	Concentration in 5ml (µg)	Cumulative concentration in 5ml (µg)	Cumulative concentration in 200ml (µg)	Cumulative % release
1 hr	0.183	0.9320	0.00466	0.00466	0.0932	9.3%
2 hr	0.281	1.4005	0.00700	0.01166	0.2232	22.3%
3 hr	0.324	1.6061	0.00830	0.01996	0.3992	39.9%
4 hr	0.486	2.3804	0.01190	0.03186	0.6372	63.72%
5 hr	0.701	3.4080	0.01704	0.04890	0.9781	97.81%

FORMULATION 2:

Table 28: *In vitro* drug release of Formulation-2

Time (hrs)	Abs (nm)	Con. in µg/ml	Concentration in 5ml (µg)	Cumulative concentration in 5ml (µg)	Cumulative concentration in 200ml (µg)	Cumulative % release
1 hr	0.326	1.615	0.0080	0.008	0.16	16%
2 hr	0.401	1.974	0.0098	0.1078	0.356	35.6%
3 hr	0.421	2.069	0.0103	0.1181	0.562	56.2%
4 hr	0.440	2.160	0.0108	0.1289	0.778	77.8%
5 hr	0.454	2.227	0.0111	0.1400	0.998	99.8%

Fig 19: *In vitro* drug release of Solasodine TDS



7.8 SKIN IRRITATION STUDIES

Table 29: Skin irritation studies in Albino rabbits

Treatment Group	On 7^{th} day	On 14^{th} day
Group I	0	0
Group II	0	0

7.9 INVIVO EVALUATION FOR ANTI INFLAMMATORY, ANALGESIC AND ANTI PYRETIC ACTIVITY

7.9.1. ANTI INFLAMMATORY EFFECT OF TRANSDERMAL PATCHES CONTAINING ISOLATED COMPOUND OF SOLASODINE

Anti inflammatory efficacy of transdermal patches of solasodine was determined on carrageen induced paw oedema and the results are tabulated.(Table 30)

Treatment group	0 hour	1 hour	2 hour	3 hour	4 hour	% inhibition at 4th hour
Control (10mg/kg)	0.0072 ±0.001	0.0072 ±0.001	0.028 ±0.002	0.024 ±0.005	0.021 ±0.005	12.5%
Indomethacin (5mg/kg)	0.0072 ±0.011	0.0072 ±0.002	0.021 ±0.005	0.021 ±0.001	0.010 ±0.005	52.3%
Solasodine TDS (30mg/kg)	0.0081 ±0.005	0.0082 ±0.002	0.024 ±0.005	0.017 ±0.002	0.010 ±0.002	57.1%

Note: The results are given as mean ± standard deviation; $p < 0.01$ compared to control (n=6)

7.9.2 ANALGESIC EFFECT OF TRANSDERMAL PATCHES CONTAINING ISOLATED COMPOUND OF SOLASODINE

Analgesic effect of transdermal patches of solasodine was studied using writhing method and the results are tabulated. (Table 31)

Treatment group	Average number of writhings/ 15 min	%inhibition
Control (10ml/kg)	55.5±2.12	0 %
Standard (Aspirin 100mg/kg)	48±1	13.5%
Solasodine TDS (30mg/kg)	41±1	26.1%

Note: The results are given as mean ± standard deviation; $p < 0.01$ compared to control (n=6)

7.9.3 ANTI PYRETIC EFFECT OF TRANSDERMAL PATCHES CONTAINING ISOLATED COMPOUND OF SOLASODINE

Anti pyretic effect of transdermal patches of solasodine was studied and the results are tabulated. (Table 32)

Treatment group	Initial rectal temper-ature (˚C)	Rectal temperature in ˚C after 18 hrs of yeast injection						% Reduction in temperature after 4 hour
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	
Control (10ml/kg)	35.6 ±1.02	37.2 ±0.42	37.8 ±0.92	37.8 ±0.84	37.6 ±0.94	36.9 ±0.92	36.7 ±0.70	1.3 %
Standard (Aspirin 100mg/kg)	35.3 ±0.42	37.7 ±2.34	37.8 ±1.13	37.7 ±1.27	37.5 ± 1.20	36.5 ±0.28	36.15 ±0.49	4.1 %
Solasodine TDS(30mg /kg)	35.8 ±0.42	37.4 ±1.34	37.6 ±1.13	37.15 ±0.94	37.1 ±0.56	36.5 ±0.28	35.8 ±0.28	4.2%

Note: The results are given as mean ± standard deviation; $p < 0.05$ compared to control (n=6)

Fig 20: Anti inflammatory efficacy of transdermal patches of solasodine using carrageen induced paw oedema technique.

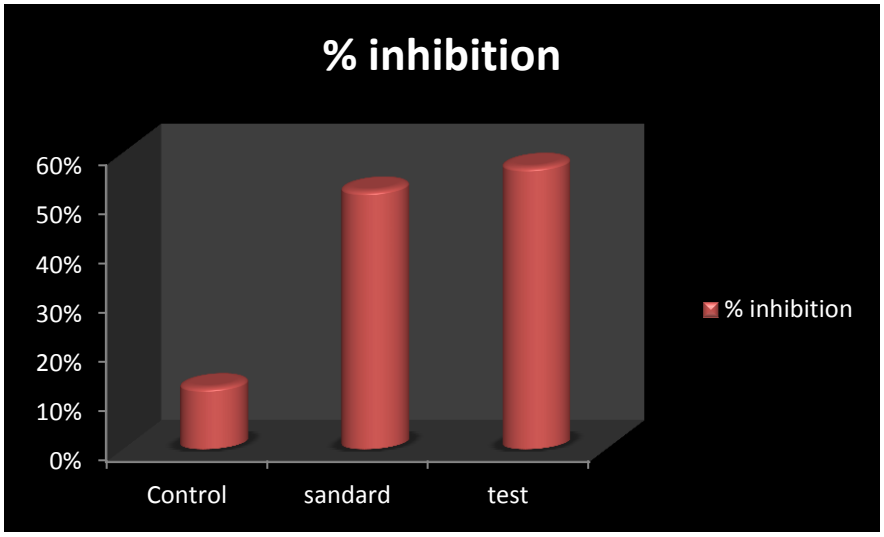


Fig 21: Analgesic effect of transdermal patches of solasodine was studied using writhing method in rats.

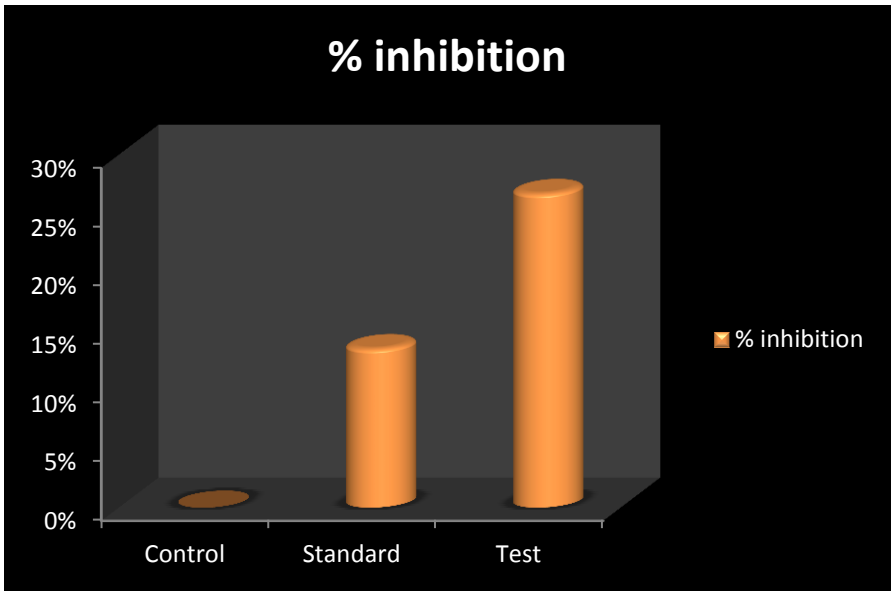


Fig 22: Anti pyretic effect of transdermal patches of solasodine

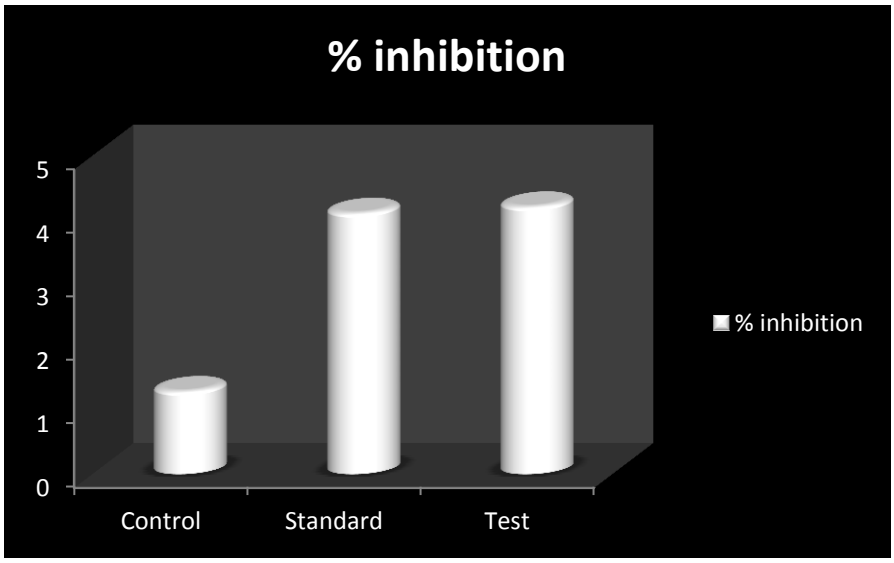


Fig 23: Isolated Solasodine from *Solanum surattense*

Fig24: TLC of Isolated compound

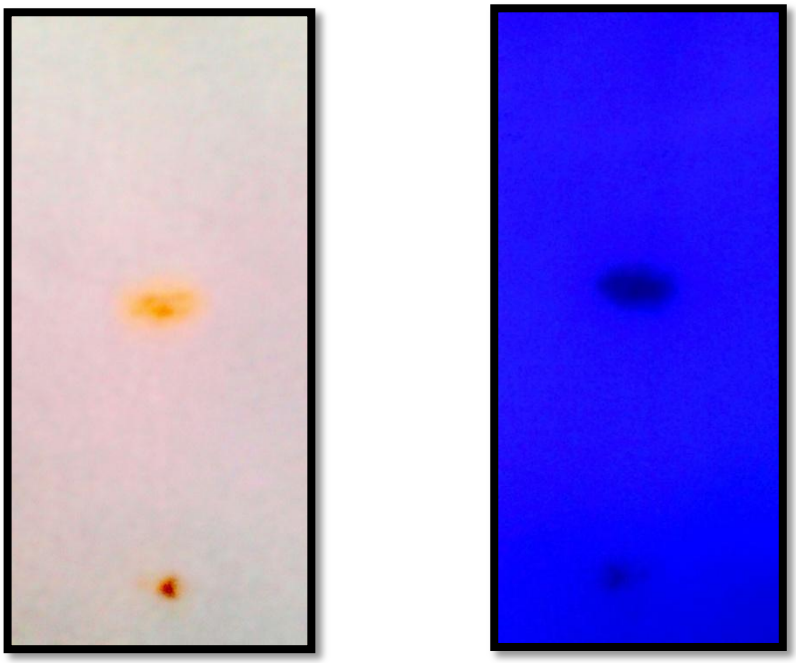


Fig 25: Formulation of Transdermal patches using Solasodine

Fig 26: Solasodine Transdermal patches

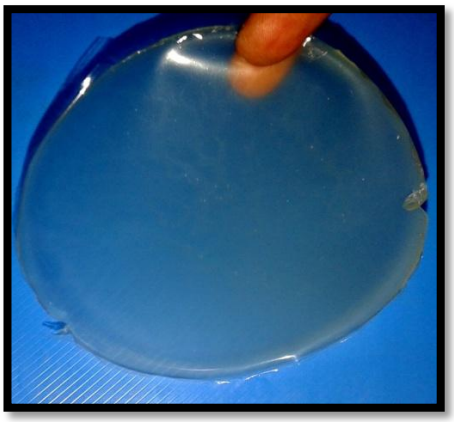


Fig 27: Skin irritation test for solasodine patches performed in rabbits

Fig 28: Paw edema in rats were performed by mercury displacement technique using Plethysmometer.

Fig 29: Rat paw before treatment

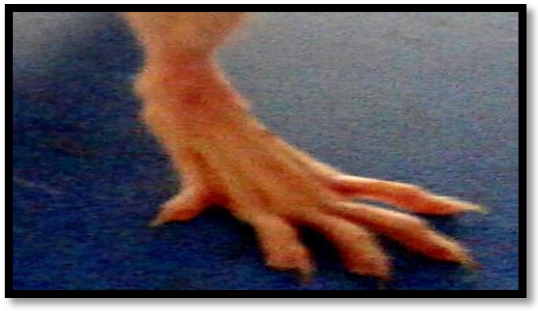


Fig 30: Animal treated with indomethacine at the end of 4th hr

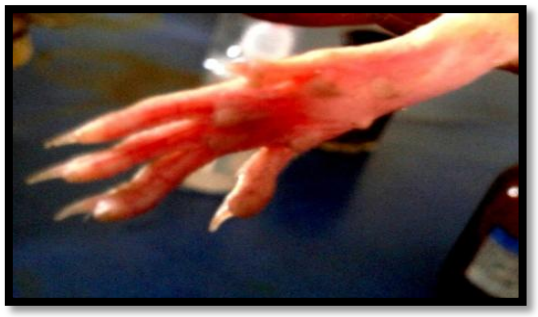


Fig 31: Animal treated with solasodine TDS at the end of 4th hr

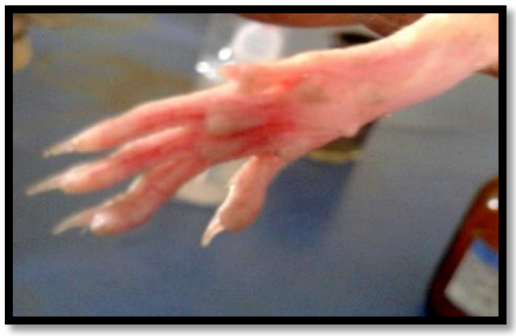


Fig 32: Rectal temperature of the animal was measured using digital thermometer

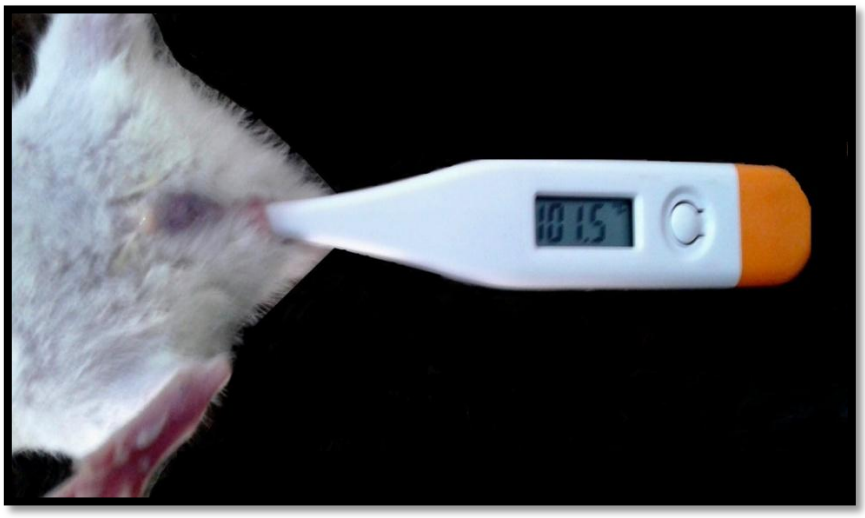


Fig 33: Mice treated with solasodine TDS were used for evaluation of analgesic activity

8. SUMMARY

The present study entitled "FORMULATION AND EVALUATION OF TRANSDERMAL PATCHES USING ISOLATED SOLASODINE FROM *Solanum surattense* FOR ANTI-INFLAMMATORY, ANALGESIC AND ANTI-PYRETIC ACTIVITY" comprises of the results of standardization parameters, formulation and evaluation of Transdermal patches and pharmacological studies broadly. The leaves and berries of *Solanum surattense* authenticated and preliminarily standardized according to Ayurvedic pharmacopeia of India and standard references. The formulated paches were analyzed for various parameters and standard values were defined for each parameter.

Physiochemical constants like loss on drying, total ash, acid insoluble ash values were determined. These values were found to be not more than **12%, 9%, 3%** respectively.

Extractive values comprised of water soluble extractive, ethanol soluble extractive, hexane soluble extractive, ethyl acetate soluble extractive were found to be not less than **16%, 6%, 9%,19%** respectively.

The Solasodine was isolated from leaves and berries of *Solanum surattense*. Isolated solasodine was subjected to TLC studies, quantitative estimation by HPTLC, Infra-red spectroscopy, NMR and MASS Spectroscopy. The R_F value of isolated solasodine was found to be 0.62 which complies with the RF value of standard. The structure of isolated solasodine was confirmed by spectral studies.

Acute toxicity studies were done according to OECD Guidelines and LD_{50} value was calculated. Solasodine up to 300mg does not show any toxicity and hence one tenth of its dose was selected for *invivo* studies.

Transdermal patches are formulated using isolated solasodine using two different polymers and patches were evaluated. Results obtained from the evaluation using various parameters are as follows.

Uniformity in weight : 0.0159 to 0.0160g

Percentage moisture absorption : 2.4155 to 2.4590 %w/w

Percentage moisture loss : 1.201 to 1.207 %w/w

Surface pH : 6

Folding endurance : >200

Percentage Drug content : 92.9 to 98.7%

Invitro drug release studies using Franz diffusion cell were done and formulation 2 which shows maximum drug release of 99.8% was selected for further *invivo* studies.

Invivo skin irritation test for transdermal patches were carried out in rabbits and shows no signs of erythema or edema at the end of 14^{th} day.

In pharmacological studies, the Solasodine TDS shows better and improved anti-inflammatory
(**57.1 %**), analgesic (**26.1 %**) and anti-pyretic activity (**4.2 %**), compared with standard indomethacin and aspirin which shows (**52.3, 4.1, 13.5 %**) inhibition of inflammation, pyrexia and pain respectively.

Objectives of the study include:

- To standardize the crude drug used for the formulation.
- Isolation of solasodine from *S.Surattense*.
- Characterization of isolated compound.
- Acute toxicity studies.
- Formulation of transdermal patches using isolated compound of solasodine.
- Standardization and evaluation of transdermal patches.
- *In vitro* evaluation of solasodine patches.
- Skin irritation test for transdermal patches in rabbits.
- *In vivo* evaluation for anti-inflammatory, anti-pyretic and analgesic activity.

9. CONCLUSION

The present study was an attempt to formulate and evaluate the transdermal patches using isolated solasodine for anti-inflammatory, anti-pyretic and analgesic activity.

The crude drug was consistent with various identity, quality and purity parameters such as physico-chemical parameters, fluorescence analysis, and heavy metal analysis. The crude drug was used for the isolation of solasodine and formulation of transdermal patches. The patch which shows maximum drug release in *in vitro* studies were selected for pharmacological evaluation.

Transdermal patches containing solasodine shows better anti-inflammatory, anti-pyretic and analgesic activity when compared to synthetic standards used such as indomethacin and aspirin.

Thus these patches may be beneficial for the treatment of inflammations, pain, fever, but detailed preclinical and clinical studies are required to establish the use of solasodine transdermal patches as anti-inflammatory, analgesic and anti-pyretic formulation.

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