Formulation & Pharmacological Evaluation Of Herbal Gel Of Pothos Scandens Linn

**Corresponding Author:**
Mr. Tariq Sainuddin,  
Pharmacist, Al Shifa College of Pharmacy, Zains villa, Chowalloor, Kandanassery P.O , 680102 - India

**Submitting Author:**
Mr. Tariq Sainuddin,  
Pharmacist, Centennial College, 680102 - India

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Formulation & Pharmacological evaluation of herbal
Formulation & Pharmacological Evaluation Of Herbal Gel Of Pothos Scandens Linn

Author(s): Sainuddin T, K.P M

Abstract

Over the past decade, herbal medicine has become an item of global importance both medicinal and economical. Although usage of these herbal medicines has increased, their quality, safety and efficiency are serious concerns in industrialized and developing countries. Thus accurate scientific assessment has become a prerequisite for acceptance of herbal health claims. *Pothos scandens* Linn. (Family: Araceae) have a great medicinal value for its wound and burn healing properties. The plant is seen many parts of South India especially in wild areas. Traditionally the plant is used by Ayurvedic physicians of Cheruvathur, Kerala mainly for its burn healing properties. For natural product discovery the conventional approach of extraction, identification and characterization of compounds, test for desired biological activity and finally formulating in a suitable dosage forms. Ayurveda based drug discovery uses “Reverse Pharmacology” in which drug candidates are first identified based on large-scale use in population, and then validated in clinical trials. Experts say this approach can cut time from 12 to 15 years for drug discovery and are economical. By providing scientific information on medicinal plants and converting these medicinal plants into good formulations can influence its productivity, therapeutic efficiency and competitiveness in the field of medicine and pharmacy.

Introduction

80% of the world population relies on medicinal plants for their primary health care. Such herbal medicines that are easily available, cheaper, time tested and considered safer than most of modern synthetic drugs. Over 50% of the best selling pharmaceuticals in use today were derived from natural products. Plants provide a bank of rich, complex and highly varied structures, which are unlikely to be synthesized in laboratories. Furthermore, evolution has already carried out a screening process whereby plants are more likely to survive if they contain potent compounds, which deter animals or insects from eating them. These potent compounds are secondary metabolites with quite complex structures, in which most of them are biologically active compounds. It is sobering that very few plants were been fully studied and the vast majorities have not been studied at all. So a preliminary phytochemical screening of the plant is performed.

In the formulation of gel, the efficacy is often dependent on the composition of the vehicle. The ability of a drug in gel formulation to penetrate the skin and exert its effect depends on to consecutive physical events. The drug must first diffuse out of the vehicle to the skin surface and then, it must penetrate the natural barrier to enter into the site of action. Carbopol polymers are bearing very good water sorption property. They swell in water up to 1000 times their original volume and 10 times their original diameter to form a gel when exposed to a pH environment above 4.0 to 6.0. Because the pKa of these polymers is 6.0 to 0.5, the carboxylate moiety on the polymer backbone ionizes, resulting in repulsion between the native charges, which adds to the swelling of the polymer. The glass transition temperature of Carbopol polymers is 105°C (221°F) in powder form. However, glass transition temperature decreases significantly as the polymer comes into contact of water. The polymer chains start gyrating and radius of gyration becomes increasingly larger. Macroscopically, this phenomenon manifests itself as swelling.

Wound healing is a natural restorative response to tissue injury. Healing is the interaction of a complex cascade of cellular events that generates resurfacing, reconstitution, and restoration of the tensile strength of injured skin.

Biological Methods for the Study of Wound Healing

Animal models and in-vitro assays have become indispensable tools for researchers in nearly every scientific discipline. In product development there is a
In-vitro studies help determine which concentrations may be effective in-vivo and determine whether certain products are effective on various cell types (e.g. fibroblasts and keratinocytes). The next step is to examine the effect of the product’s use in an animal model(s). This facilitates investigation of the product in the presence of wound fluid, blood, immune cells, proteases, etc., which can have an effect on the activity of the active agent. Many in-vivo animal studies initially investigate the safety and/or irritancy of the product. It is important to be sure that these agents do not have a toxic effect on tissues. Efficacy animal trials are conducted after the safety studies are completed. This eventually allows the product to be evaluated in human trials. Although definitive studies conducted on human subjects are needed, such studies present several practical, ethical, and moral concerns. For example, in order to examine wounds histologically throughout the entire healing process one must biopsy a human subject at multiple time points, which is impractical. Furthermore, ethical considerations prevent the intentional infection of a wound on a human or the use of an untreated control subject. Some of the practical difficulties lie in obtaining enough subjects with similar or identical situations to conduct well controlled studies. Another complication to factor in with human trials is compliance (e.g. subject’s level of cooperation, ability to understand and follow instructions). The above difficulties have led researchers to develop multiple in-vitro and in-vivo models that attempt to mimic or reproduce human conditions.

[1] In-vitro technique

In-vitro assays are great for examining the effect of agents on particular cell types. They are relatively inexpensive, fast, and convenient for the researcher. In addition to providing useful results in a short time, they possess an obvious humane appeal since they usually do not involve the use of animals or humans. In-vitro assays are useful in wound healing research for determining the possible effectiveness of various treatments, particularly antimicrobial and healing enhancing agents. Another noteworthy attribute of in-vitro testing is the ability to screen multiple agents or samples simultaneously. Assays can aid in the early detection of antimicrobial resistance among pathogens and determination of minimal inhibitory concentrations (MIC), and allow for highly specific control over the experimental conditions. However, it is difficult to simulate a “real world” application. Although some variables such as pH, salinity, and temperature are easily controlled, in-vitro assays are incapable of completely reproducing biological conditions (e.g. immune responses, healing) and diseases, such as diabetes.

In order to approximate in-vivo experiments, in-vitro assays have been developed that incorporate some variety of cell or tissue system. Wound closure studies have been conducted on single cell monolayer systems. The principle in vitro technique for studying the skin penetration evolves the use of variety of diffusion cells in which animal or human skin is fastened to a holder and the passage of compounds from epidermal surface to a fluid bath is measured. Many chemical agents can be used which penetrate in sufficient concentration to be determined by different physical and chemical analysis. More recently model systems have been used which do not use membranes. Solvent such as alcohol –water have been used as models chosen to have negligible solubility in phase representing the skin, but in which drug is fairly soluble. A receptor phase like chloroform and isopropyl myristate can also be used to receive the penetrant.

Important factors influencing release in to receptor phase are solubility in the vehicle and partition coefficient of the drug between vehicle and the receptor phase. Optimum release is obtained from vehicle containing the minimum concentration of solvent required for complete solubilization of the drug.

[2] In-vivo technique

Small mammal wound healing models. Rodent and small mammal models of wound healing have emerged as the model of choice for many researchers. This type of study is beneficial to wound research for multiple reasons. Small animals are inexpensive, easily obtainable, and require less space, food, and water. Additionally, they often have multiple offspring, which develop quickly allowing experiments to proceed through multiple generations. Small animals usually have accelerated modes of healing compared to humans, thus experiment duration lasts for days, as opposed to weeks or months in human experiments. Some small mammals can easily be altered genetically and provide a wound model capable of approximating defective human conditions such as diabetes, immunological deficiencies, and obesity. Another advantage of small mammal models is their ability to serve in experiments where death is an endpoint, as is some cases of bacterial or viral infection.

Small animals provide a multitude of model choices for
various human wound conditions. Some models have been developed to investigate the mechanistic particulars of certain aspects of healing. The major in-vivo methods are histological techniques, use of tracers, analysis of body fluids and tissues and elicitation of biological response. Tissue changes in skin following the application of various substances to the cutaneous surface can yield information about specific tissues affected, so that not only absorption is revealed but also the route of penetration. For studying the wound in the laboratory, mainly two types of wounds are produced experimentally. These are excised or open wounds and incised or sutured wounds. The assessment of healing is made by studying the regenerating tissue by different parameters. Following types of wounds are made in laboratory animals for studying the effect of various drugs.

1) Excised wound or Open Wound
These types of wounds are prepared either on rats or guinea pigs. Back of each animal is shaved and prepared after washing with spirit for operation. An area of about 2.0 sq cm is marked out by an Indian market ink with the help of stencil. The marked area is excised with sharp knife and scissors under ether anesthesia. After making wound the animals are divided in two groups. One control group and the other test group and are kept in isolated cages. Topical application of ointments or lotions are made on is founds daily. On desired postoperative days this founded animals are sacrifice and the contraction measured. Biochemical estimation of granulation tissue and histological examination's are done.

2) Incised Or Sutured Skin Wounds
After preparing the animals for operation under aseptic conditions, a longitudinal cutaneous incision measuring about 3-5c.m was made at the back or abdomen according to the type of animals selected. Wounds are closed by interrupted cotton threads stitches, which are placed approximately at equal distance. The tensile strengths, biochemical and histological study of the wound are carried out.

3) Musculoperitoneal Wounds
To prepare this wound, animals are prepared in the same fashion as described earlier but their abdomen is opened completely incision measuring between 2-5c.m according to the size of the animals are made. The wound is caused in one layer by interrupted linen stitches, which are placed approximately at equal distance. The tensile strengths, biochemical and histological study of the wound are carried out.

4) Burn wounds
The burn is produced under aseptic condition on hair removed areas of back of rats/guinea pigs with special device cosseting of a square sheet of an iron piece measuring 4.8sq.cm with wooden handle. It is heated to a red hot over flying and is placed in contact with the back of the anaesthetized rat up to ten seconds, with out any pressure. Medication is applied these animals are sacrificed on desired days and the regenerated tissues are removed for biochemical and the histological studies etc. their wounds are also measured for the contraction.

5) Dead Space Wound Method
Subcutaneous implantation of sterilize cotton pellets (10 mg each) and a plastic road (25-30mm) in the axial are anti groin respectively is done under ether anesthesia in male albino rabbits. The 10th day old granulomas are carefully dissected and cleared of the tissues.

METHOD OF ASSESSMENT

1. MACROSCOPIC EXAMINATION OF THE WOUNDS:
Gross examination of wound gives some information regarding the healing. One can easily distinguish the normally healing wounds with that of a wound with delayed healing by a careful gross examination provided the different is marked in both the wounds. Gross examination sometimes may not give much information; hence quantitative methods can be used in such cases. For this purpose measurement the size of the wounds gives sufficient information. This can be done using a planimeter or using a graph paper. Method used in this project is Macroscopic Examination.

2. MICROSCOPIC METHOD
This method involves histological examination of tissue.

3. ELECTRON MICROSCOPIC METHOD
This technique is used for the study of details about the cellular morphology and other alteration at cellular levels during healing and regeneration.

Plant Profile & Literature Review

Plant Profile9 : Pothos scandens (Araceae)
BOTANICAL INFORMATION9
Botanical Name : Pothos scandens
Division : Magnoliophyta
Class : Liliopsida
Subclass : Monocots
Order : Alismatales
Family : Araceae
Subfamily : Pothoideae
Tribe : Potheae

Vernacular Names
Malayalam : Annaparuvu, Paruvakodi
Tamil : Anaparuga
Kannada : Adkebiluballi

Botanical Description:
Pothos scandens is the botanical name of the plant. It is a climbing shrub having adventitious aerial roots. The internodes of the plant are 1.3-2.5 cms and its leaves are very variable. The leaves are obovate, elliptic or lanceolate and coriaceous, having a bright green colour.

The apex of the plant is acute, acuminate or apiculate, with cuneate or rounded base. The petioles of Pothos scandens are semi-amplexicaul and broadly winged. They have a length of 2.5-7.5 cms and a width of 0.6-1.7 cm at the base. The green Spathe is 0.4-0.7 cm long, ovate and erect, with cuspidate apex. The stipe of the plant is deflexed, to 0.6 cm long and the spadix is yellow, with an approximate length of 0.5 cm. The spadix is globose, ovoid or shortly oblong. The fruits or berries of the plant are oblong and 1.3-1.7 cm long and they are scarlet when ripe.

Geographical Source:
AFRICA : Western Indian Ocean: Comoros; Madagascar; Seychelles ASIA-TEMPERATE: China: China
ASIA-TROPICAL: Indian Subcontinent: Bangladesh; India - Assam, Bihar, Goa, Karnataka, Kerala, Maharashtra, Meghalaya, Orissa, Tamil Nadu, Tripura, West Bengal, Andaman and Nicobar. Others: Cambodia, Myanmar, Thailand, Vietnam Indonesia

Ethnobotanical Uses: Pothos scandens has quite a few medicinal properties and usages. The bruised root of the plant is reportedly applied to promote healing of abscesses, after being fried in oil. The Indian people use an infusion of the leaves of this plant as a bath for curing convulsions and epilepsy. Apart from that, the stem is also reportedly used to treat asthma, after being cut up with camphor and smoked like tobacco. Traditionally the plant is used by Ayurvedic physicians of Cheruvathur, Kerala mainly for its burn healing properties. Other uses include in treatment of vermiﬁge and smallpox.

Review of Literature 11

S. Ignacimuthu et al studied the traditional knowledge of Kani tribals in Kouthalai of Tirunelveli hills, Tamil Nadu, India. An ethnobotanical survey was carried out among the ethnic groups (Kani/Kanikaran) in Southern Western Ghats of India. Traditional uses of 54 plant species belonging to 26 families are described under this study including Pothos scandens. In this communication, the information got from the tribal was compared with the already existing literature on ethnobotany of India. The documented ethnomedicinal plants were mostly used to cure skin diseases, poison bites, wounds and rheumatism. The medicinal plants used by kanis are arranged alphabetically followed by family name, local name, major chemical constituents, parts used, mode of preparation and medicinal uses. Christine A. Williams et al studied the Anthocyanin pigments and leaf flavonoids in the Family: Araceae. The study revealed that Anthocyanins, variously identified in inflorescence, fruit, leaf or petiole of 59 representative species of the Araceae, are of a simple type.

Mohsin Raza et al studied the anticonvulsant activities of 334 medicinal plants used for the treatment of epilepsy and convulsive disorders in the indigenous system of medicine including Pothos scandens. Geoffrey C. Kite et al studied the Polyhydroxyalkaloids in the Aroid Tribes Nephthytideae and Aglaonemateae. They conducted a survey of polyhydroxyalkaloids in species of 52 genera of Araceae revealed the presence of 2,5-dihydroxymethyl-3,4-dihydroxyphenyl fraction (DMDP) and α-homonojirimycin (HNJ).

S.A. Salgare et al studied the effect of Ambient Air (from Chembur) on the Chlorophyll Content of Cultivated Plants. The ambient air from Chembur inhibited the chlorophyll content of plants collected from polluted zones. The plants were collected from three different zones i.e. Collector’s colony, Chembur Colony and Colaba (treated as control). Collections were made in the winter season. The plants for this study are Malvaviscus arboreus, Graptophyllum bertense. Ixora cocceinea, Nerium odorum, Pothos scandens, Quisqualis Indic, Tanbernae Montana coronari. Chlorophyll was estimated using Arnon’s method. Maximum inhibition in the chlorophyll content was found with plants collected from Collector’s colony. Grewal,-J-S12 investigated the biochemical factors responsible for susceptibility or resistance of various plants against the scarlet mite, Brevipalpus phoenicis: I. amino acids analysis. Results are presented of amino acid analysis by thin layer chromatography for 11 out of 31 plant species screened for resistance or susceptibility to infestation by Brevipalpus phoenicis. Species stated to be resistant (Pothos scandens, Bauhinia variegata, Eucalyptus globulus and maize) contained tryptophan, tyrosine and hydroxyproline. Plant species lacking dihydroxyphenylalanine (Vicia feba [faba beans], Dalbergia sissoo and Cestrum nocturnum) did not support the development of B. phoenicis

Dhanavel,-D13 et al conducted Cytotaxonomical studies in South Indian Araceae. Studies were carried...
out in 27 species belonging to 15 genera of Araceae from Tamil Nadu, India. First record of chromosome numbers were made in 10 species, namely Alocasia macrorrhiza var. dark pink (2n=28), Pothos scandens (2n=32), Anthurium cubense (2n=30), A. polyrrhizum [A. polyrrhizon] (2n=16), Caladium bicolor var. local (2n=24), C. bicolor var. white stick with red spot (2n=40), Philodendron cymbispathum (2n=36), P. mello-baretoanum [P. mello-barretoanum = P. bipinnatifidum] (2n=30), Spathiphyllum wallisii (2n=18) and Dieffenbachia amoena (2n=54). The somatic chromosome number (2n) ranged from 16 to 54. The primary basic chromosome number may be 8 and other basic numbers should have originated by the addition of one or few basic chromosome numbers. The karyotype analyses show that each genus and species of a particular genus has a particular combination of different types of chromosomes. Therefore, karyotype alteration of chromosome play an important role in speciation, along with aneuploidy, euploidy and higher polyploidy. Hence, the present study of interrelationship among them will be more useful for future breeding programmes.

Plan of Work

PHARMACOGNOSTICAL STUDIES
The Phytochemical investigations of a plant involve the following:
Authentication of the plant
Determination of Physicochemical Parameters
Extraction of the plant (Ethanol & Aqueous Extract)
Isolation and Characterization

PHARMACEUTICAL STUDIES
1. Formulation with Carbopol 940 in different concentration to obtain a stable gel
2. Evaluation of Carbopol 940
Physical Observation
Estimations of Drug Content
Extrudability
pH determination

PHARMACOLOGICAL STUDIES
Primary Skin Irritation Test
Primary irritation test was done on rats by placing a piece of cotton wool soaked in a saturated solution of ethanolic extract of Pothos scandens on a shaved portion of dorsal skin and securing it firmly in place with adhesive plaster. This was allowed to remain in close contact with the skin for 24 hours, after which the site of application was examined for irritation with 0.8% formalin as control.

Wound Healing Studies
Healthy male albino rats was selected (150-250), from Kerala Agriculture University, Mannuthy, Thrissur were used. The animals are kept in cage for 20 days well fed. The back of the animal was shaved and washed with spirit. A circular area of 1cm diameter was marked with a marker on either side of bump region. The animals were anaesthetized with a combination of ketamine and xylazine. The back of the animal was shaved and washed with spirit. A circular area of 1cm diameter was marked with a marker on either side of bump region. A trichotomy of the back of the rats was performed, sufficient for 2 perforations are made (test and control). The pieces of tissue were subsequently excised with aid of scissors, scalpels and a forceps. The wounds on the left side were filled with gel extract (test wounds) and on the right side (control wounds) were filled with alcohol. After these procedures, the animals receive no other treatment until they are fully recovered. The application was received daily for the next 24 hours post operative days.
The wound contractions were measured as percentage reduction in wound area for the 4th, 8th, 10th, and 12th days. The progressive decrease in the wound area was monitored periodically by tracing the wound margin on a tracing paper and area is accessed by placing a graph paper over a tracing paper.

Result & Discussion

Pharmacognostical studies
1. PHYSICOCHEMICAL PARAMETER
Physicochemical parameter like extractive values were determined for the selected plant material the result are shown in table No: 1. Data showing extractive value of the leaves of Pothos scandens Linn

2. PRELIMINARY PHYTOCHEMICAL SCREENING
90% Ethanol extract & Aqueous extract of the fresh leaves of Pothos scandens was subjected to Phytochemical screening

PHARMACEUTICAL STUDIES
FORMULATION TRIAL
The given polymer concentration of 0.5, 1.0, 1.5 and 2.0%, the gel consistency in 0.5% was less when compared to higher polymer concentration.

EVALUATION OF GELS Pothos scandens ALCOHOLIC EXTRACT WITH CARBOPOL 940

PHYSICAL OBSERVATION
Primary Skin Irritation Test
There was no sign of any kind of reaction, thus the ethanolic extract of Pothos scandens was found to be safe.
WOUND HEALING STUDIES
Excision wound healing studies showing percentage reduction in wound size in rats (% closure)

Conclusion

The plant Pothos scandens was selected for the study, whose extract was very useful in the treatment of wounds. Literature survey revealed that this plant is used traditionally for various ailments, especially for its wound healing property. Extensive scientific studies were not performed on this plant. Its wound healing property was not undertaken for any scientific study. Hence the present work is performed. From the present study entitled “FORMULATION & PHARMACOLOGICAL EVALUATION OF HERBAL GEL OF Pothos scandens” the following conclusions could be drawn.

Physicochemical parameters both alcohol-soluble and water-soluble extractive values were determined and the results were tabulated in Table No.1

Preliminary phytochemical studies of 90% ethanolic extract were found to contain alkaloids, protein and flavanoid. Aqueous extract contain carbohydrate, protein, flavanoid and the results were tabulated in Table No. 2

Different gel formulations of the ethanolic extract were prepared using Carbopol 940 in varying proportions of 0.5, 1.0, 1.5 and 2.0%. On physical evaluation the gel consistency of Formulation A1 was less when compared to Formulation A2, A3, and A4. Formulation A3 and A4 were found to be translucent. But formulation A2 was found to be transparent, non greasy and stable. The PH of the formulation ranges from 6.8 to 7.6 and had an excellent Extrudability and the results were tabulated in Table No. 3. Hence Formulation A2was selected for further study.

Primary Skin Irritation test were performed for Formulation A2and there was no signs of irritation. As no relevant data was available regarding the dose of topical application of the formulation, wound healing studies were carried out. Wound healing took place on 14th day in case of Test formulation and control has taken additional six days for complete wound healing. The results were shown in Table 4 (Test & Control)as percentage contraction of wounds in Rats. Therefore, the given formulation of ethanolic extract of the plant Pothos scandens was affective in wound healing.

Reference

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8. Udupa, K.N, Advances in Research in Indian Medicine, B.H.U, Publishers, Page No: 270-303
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Illustrations

Illustration 1

Tables

Table No: 1

<table>
<thead>
<tr>
<th>S.No</th>
<th>Plant material</th>
<th>Extractive value (%w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Alcohol Soluble</td>
</tr>
<tr>
<td>1</td>
<td>Fresh leaves of <em>Pothos scandens</em></td>
<td>19.32%</td>
</tr>
</tbody>
</table>

Table No: 2

<table>
<thead>
<tr>
<th>S.N</th>
<th>TESTS</th>
<th>90% ethanolic extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ALKALOIDS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Dragendorff’s Test</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>b.</td>
<td>Wagners test</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>c.</td>
<td>Hagers test</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>d.</td>
<td>Mayer’s Test</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>2</td>
<td>CARBOHYDRATES</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Molish test</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>b.</td>
<td>Iodine test</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>c.</td>
<td>Fehling’s test</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>d.</td>
<td>Benedict’s test</td>
<td>-ve</td>
<td>+ve</td>
</tr>
</tbody>
</table>
It has been found that the 90% alcoholic extract contain alkaloids, proteins and flavanoids. Aqueous extract contain alkaloids, carbohydrates, proteins and flavanoids.

<p>| | | |</p>
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<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>a.</td>
<td>Libermann sterol test</td>
<td>-ve</td>
</tr>
<tr>
<td>b.</td>
<td>Libermann-Butchard test</td>
<td>-ve</td>
</tr>
<tr>
<td>c.</td>
<td>Salkowsky test</td>
<td>-ve</td>
</tr>
<tr>
<td>6.</td>
<td>AMINO ACIDS &amp; PROTEINS</td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Biuret test</td>
<td>+ve</td>
</tr>
<tr>
<td>b.</td>
<td>Xanthoproteic test</td>
<td>+ve</td>
</tr>
<tr>
<td>c.</td>
<td>Ninhydrin test</td>
<td>-ve</td>
</tr>
<tr>
<td>7.</td>
<td>TANNINS &amp; PHENOLICS</td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>K$_2$Cr$_2$O$_7$ test</td>
<td>-ve</td>
</tr>
<tr>
<td>b.</td>
<td>FeCl$_3$</td>
<td>-ve</td>
</tr>
<tr>
<td>c.</td>
<td>Lead acetate</td>
<td>-ve</td>
</tr>
<tr>
<td>8.</td>
<td>FLAVONES &amp; FLAVONONES</td>
<td></td>
</tr>
<tr>
<td>a.</td>
<td>Aqueous NaOH</td>
<td>+ve</td>
</tr>
<tr>
<td>b.</td>
<td>Shinoda Test</td>
<td>+ve</td>
</tr>
<tr>
<td>9.</td>
<td>FIXED OILS</td>
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</tr>
<tr>
<td>a.</td>
<td>Spot Test</td>
<td>-ve</td>
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<tr>
<td>b.</td>
<td>Saponification Test</td>
<td>-ve</td>
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<tr>
<td>pH Measurement</td>
<td>A1</td>
<td>A2</td>
</tr>
<tr>
<td>----------------</td>
<td>----</td>
<td>----</td>
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<td></td>
<td>7.0</td>
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<tr>
<th>EXTRUDABILITY</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>A4</th>
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<tbody>
<tr>
<td></td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</table>

++ Good
+++ Excellent

**TABLE-4**

**TEST(ETHANOLIC EXTRACT)**

<table>
<thead>
<tr>
<th>DAYS</th>
<th>R_A</th>
<th>R_B</th>
<th>R_C</th>
<th>R_D</th>
<th>R_E</th>
<th>R_F</th>
<th>AVG</th>
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<tbody>
<tr>
<td>4TH</td>
<td>20%</td>
<td>22.42%</td>
<td>17.11%</td>
<td>19.92%</td>
<td>20.46%</td>
<td>18.46%</td>
<td>19.73%</td>
</tr>
<tr>
<td>8TH</td>
<td>43.16%</td>
<td>56.93%</td>
<td>30.80%</td>
<td>32.48%</td>
<td>31.96%</td>
<td>28.43%</td>
<td>37.29%</td>
</tr>
<tr>
<td>10TH</td>
<td>75.53%</td>
<td>87.18%</td>
<td>53.23%</td>
<td>59.49%</td>
<td>53.26%</td>
<td>57.85%</td>
<td>67.42%</td>
</tr>
<tr>
<td>12TH</td>
<td>83.62%</td>
<td>90.2%</td>
<td>75.29%</td>
<td>80.29%</td>
<td>82.13%</td>
<td>88.76%</td>
<td>83.38%</td>
</tr>
</tbody>
</table>
On the first day after the excision of skin wounds made at an area of approximately 274mm² and its macroscopical studies were performed as percentage decrease in wound size.

<table>
<thead>
<tr>
<th>DAYS</th>
<th>R_A</th>
<th>R_B</th>
<th>R_C</th>
<th>R_D</th>
<th>R_E</th>
<th>R_F</th>
<th>AVG</th>
</tr>
</thead>
<tbody>
<tr>
<td>4TH</td>
<td>10.23%</td>
<td>9.76%</td>
<td>11.14%</td>
<td>12.28%</td>
<td>10.87%</td>
<td>13.73%</td>
<td>11.34%</td>
</tr>
<tr>
<td>8TH</td>
<td>17.87%</td>
<td>16.23%</td>
<td>19.23%</td>
<td>20.71%</td>
<td>16.58%</td>
<td>17.55%</td>
<td>18.03%</td>
</tr>
<tr>
<td>10TH</td>
<td>29.23%</td>
<td>27.12%</td>
<td>26.78%</td>
<td>27.25%</td>
<td>27.44%</td>
<td>25.43%</td>
<td>27.21%</td>
</tr>
<tr>
<td>12TH</td>
<td>34.47%</td>
<td>32.21%</td>
<td>35.46%</td>
<td>33.33%</td>
<td>34.86%</td>
<td>84.67%</td>
<td>34.17%</td>
</tr>
</tbody>
</table>

\( R_A, R_B, R_C, R_D, R_E \) - Designation for each rats

\( C \) - Control \hspace{1cm} \( T \) - Test

Average Percentage contraction of wounds in rats after treating with Carbopol 940

On the first day after the excision of skin wounds made at an area of approximately 274mm² and its macroscopical studies were performed as percentage decrease in wound size.

On the forth day, the average percentage decrease in wound size in test was found to be 19.73% and that of the control 11.34%

On the eight day, the average percentage decrease in wound size in test was found to be 37.29% and that of the control 18.03%

On the tenth day, the average percentage decrease in wound size in test was found to be 64.42% and that of the control 27.21%

On the twelfth day, the average percentage decrease in wound size in test was found to be 83.38% and that of the control 37.17%

It was found that complete wound healing for the Test Formulation took place on 14th day and for the control has taken additional six days for the complete wound healing. So the given formulation of ethanolic extract of the plant Pothos scandens was affective in wound healing.
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