ejpmr, 2018,5(3), 1-10

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

FORMULATION AND EVALUATION OF POLYHERBAL TRANSDERMAL GEL FOR ANTI-FUNGAL ACTIVITY

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Article Received on 24/12/2017 **Article Revised on 14/01/2018** **Article Accepted on 03/02/2018**

ABSTRACT

Conventional drug delivery system has many problems so bulk of research has now shifted from synthetic drugs to herbal drugs. This is possible because of the vast variety of bioactive molecules in the plants and their higher safety margin. Now a days there is a greater global interest in non synthetic, natural drugs derived from plant/herbal sources due to better tolerance and minimum adverse drug reactions. Herbal drugs, used in Indian systems of medicine are however claimed to be effective and safe. Phytotherapy is an alternative for fungal infection control; different species of plants are used in folk medicine. Around the world, different species play an important role on fungal infection process, for example *Cassia alata* is used in the treatment of fungal infection, in other studies showed the anti-fungal potential of *Cedar* in *external* route. The genus *lemon grass oil, coleus aromaticus oil, Aloe vera* gel constitutes an important source of Citral, Geraniol and Terpenoids these compounds are used for the fungal infection. Based on the present study, it can be concluded that polyherbal extracts possesses anti-fungal activitiy. But by doing comparision studies maximum zone of inhibition was observed for formulation (Transdermal gel) compared to polyherbal extracts.

KEYWORDS: *Lemon Grass Oil, Coleus Aromaticus Oil, Aloe Vera* Transdermal Patch.

INTRODUCTION

Herbal product is a chemical substance or compound produced from a living organisms, found in nature. Many cosmetics, food and dietary supplements produced from natural sources without adding artificial ingredients.[1] The rich content of antifungal substances in plants are being used biopesticide since up to the beginning of human civilization. Antifungal effects of plant and plant products emerge clearly every day. Antifungal substances which are obtained from plants have no side effect against environment thus, giving a significant advantage. Nowadays, a commercial pesticide used against plant diseases is found to cause damage to environment and human health. Because of that conducting a research of alternative control methods comes into prominence for minimizing used commercial pesticide. Research found that compounds in the structure of plants and essential oil were showed antifungal, antibacterial, insecticidal, nematicidal, herbicidal and antiviral activities.[2,9]

FUNGAL INFECTION

The variety of fungi can affect a person's skin. When it happens the person has a fungal skin infection. Fungi disease also called as mycosis (cutaneous or superficial mycosis).The fungi live only in the dead, stratum corneum (topmost layer) and it don't penetrate deeper. Some fungal infections produce only a small amount of irritation, scaling and redness or cause no symptoms. And some fungal infections causes itching, swelling, blisters, and severe scaling.[10]

Table No.1.1: Different Fungi Responsible For Skin Infection.

S.No	Type of skin fungal infection	Responsible fungi
1	Athlet's foot	*Epidermophyton fluccosum* *Trichophyton* *T.rubrum* *T.mentagrophytes*
2	Scalp fungal infection	*Trichophyton* *Microsporum*
3	Groin & buttocks fungal infection	*Candida albicans*
4	Tinea barbae	*T.verrucosum* *T.mentagrophytes*
5	Tenia manuum(hand fungal infection)	*T.verrucosum* *Trichophyton erinacei* *Microsporum canis* *M.gypseum*
6	Fungal nail infection	*Trichophyton rubrum* *Candida albicans* *Fusarium*
7	Skin fungal infection(Ring worm)	*Trichophyton rubrum* *T.verrucosum* *Microsporum canis* *Epidermophyton floccosum*
8	Tinea versicolor	*Malassezia furfur* *Malassezia globosa*

TRANSDERMAL GEL

The Transdermal drug delivery systems (TDDS) are self contained discrete dosage forms which can applied to intact skin that delivery the drug to the skin at a control rate to the systemic circulation. The success of Transdermal drug delivery depends on the ability of drug to permeate the skin at sufficient quantities to achieve its desired therapeutic effect. Gels have better potential as vehicle to administer drug topical in comparison to ointment because they are non sticky and requires low energy during the formulation and it is stable and have aesthetic value. The term gel is semi-solid, three dimensional, polymeric matrices containing small amount of solid dispersed in relatively large amount of liquid, yet possessing more solid in character. These systems form three dimensional, polymeric matrixes in which high degree of physical reticulation has been comprised. Gel formulation provides better application property and stability in comparison to cream and ointments. Within the large group of semisolid preparations, the use of Transdermal gel has expended in both cosmetics and pharmaceutical preparations.

MATERIAL AND METHODS

COLLECTION AND AUTHENTICATION

The whole plants of three plants such as *lemon grass, Coleus aromaticus, Aloe vera* were collected from surroundings of Tirupati, India in the month of may 2014. The taxonomical identification and authentication of the plants were done by Dr. P.Jayaraman, Retd Professor, Presidency College, and Chennai. The voucher specimen is preserved in the Department of Pharmacognosy, Sree Vidyanikethan College of Pharmacy for further reference.

EXTRACTION OF PLANTS

COLEUS AROMATICUS

Isolation of Essential Oil

The fresh plant material was subjected to hydrodistillation in a Clevenger type apparatus for 5 h. The oil was collected, dried over anhydrous sodium sulfate, measured and stored at **-4^{o} C** for further analysis.[11]

LEMONGRASS

Plant Materials

The leaves of Lemongrass (*Cymbopogon citratus*) were first chopped into small pieces and kept in a dark and cold refrigerator until it used for the experiments. Before run the experiment the plant material were soaked in water based on in water to raw material ratio.

Distillation of lemongrass

Weigh 25 g of lemongrass and cut it into small pieces. The pieces were taken in 500 ml of round bottomed flask. Add 300 ml of distilled water to the flask containing the grass and set the apparatus for distillation. Boil the mixture vigorously and collect the distillate until no more oily drops can be seen passing over. More water should be added if necessary to avoid charring of flasks contents. Extract the distillate with hexane, dry them over sodium sulfate and remove the solvent on rotary evaporator with external heating at 45°C. Finally 2-3 ml of yellow to ochre coloured oily liquid with fresh lemon – like tone with a hint of rose was obtained.[12]

ALOE VERA

Extraction method

Mature, healthy and fresh leaves of *A. vera* were washed in the running tap water for 5 min and rinsed with sterile

distilled water, then dissected longitudinally and the colourless parenchymatous tissue (aloe gel) was scraped out using a sterile knife without the fibres. The gel was ground with DMSO using the mortar and pestle. The extracts were filtered using Whatman No. 1 filter paper and the filtrate was centrifuged at 5000 rpm for 5 min. The supernatant was collected and stored in refrigerator at 4°C.[13]

EVALUATION STUDIES

PHYTOCHEMICAL EVALUATION OF ALOE VERA GEL

The gel extract was subjected to chemical tests for the identification of the various Phytoconstituents as per the standard procedure.[14]

Detection Of Carbohydrates And Glycosides

Small quantity of gel extract was subjected to the following tests to the presence of carbohydrates.

Molisch test

The gel was treated with alcoholic solution of α-Naphthol and a few dropes of conc.H_2SO_4 were added through the sides of the test tube. The formation of violet ring at the junction of the liquids indicates the presence of carbohydrates.

Benedict's test

Gel was treated with 5ml of Benedict's reagent and heated on a water bath for a few min. The formation of red-orange ppt indicates the presence of reducing sugars.

Borntrager's Test

Borntrager's test is employed for presences of anthraquinones. The drug is boiled with dilute sulphuric acid, filtered and to the filtrate benzene, or ether or chloroform is added and shaken well. The organic layer is separated to which ammonia is added slowly. The ammoniacal layer shows pink to red color due to presences of anthraquinone glycosides.

Legal Test

The test is employed for digitoxose containing glycosides. The extract of drug is dissolved in pyridine, sodium nitroprusside solution is added to it and made alkaline, pink or red color is produced.

Test for alkaloids

Dragendorff"s Reagent

It was prepared by mixing solution A (17 g of bismuth sub nitrate + 200 g of tartaric acid + 800 ml distilled water) and solution B (160 g potassium iodide + 400 ml distilled water).

Above Dragendorff"s reagent was sprayed on Whatman no. 1 filter paper then the paper was dried. The test filtrate after basification with dilute ammonia was extracted with chloroform and this extract was applied on the filter paper, impregnated with Dragendorff"s reagent with the help of capillary tube. Development of an orange red color on the paper indicates the presence of alkaloids.

Mayer's Reagent

1.36 g of mercuric chloride was dissolved in 60 ml water and 5 g of potassium iodide dissolved in 10ml of distilled water, solution were mixed and diluted to make up volume 100 ml. To a little of each extract taken in dilute hydrochloric acid in a watch glass, few drops of the reagent was added, formation of cream colored precipitate shows the presence of alkaloids.

Wagner's Reagent

1.27 g of iodine and 2 g of potassium iodide were dissolved in 5 ml of water and the solution was diluted to 100 ml with water. When few drops of this reagent were added to the test filtrate, a brown color precipitate was formed indicating the presence of alkaloids.

Hager's Reagent

A saturated aqueous solution of picric acid was employed for this test. When the test filtrate was treated with this reagent, an orange yellow precipitate was formed indicating the presence of alkaloids.

Test for saponins

Foam Test

A few mg of the test residue was taken in a test tube and shaken vigorously with small amount of sodium bicarbonate and water. If stable, characteristic honeycomb like froth is obtained, saponins are present.

Test for tannins

The test residue of each extract was taken separately in water, warmed and filtered. Tests were carried out with the filtrate using following reagent-

Ferric Chloride Test

A 5% solution of ferric chloride in 90 % alcohol was prepared. Few drops of this solution was added to a little of the above filtrate. If dark green or deep blue color is obtained, tannins are present.

Lead Acetate Test

A 10 % w/v solution of basic lead acetate in distilled water was added to the test filtrate. If precipitate is obtained, tannins are present.

Test for flavonoids

Schinoda Test

A small quantity of test residue was dissolved in 5 ml of ethanol (95% v/v) and treated with few drops of concentrated hydrochloric acid and 0.5 g of magnesium metal. The pink, crimson or magenta color is developed within a minute or two, if flavonoids are present.

PHYSICAL EVALUATION OF OILS

Odour

The Volatile oils must possess their characteristic natural odour. An expert can judge the quality of the oil and can

differentiate between adulterated, artificial and genuine oils. The odour can be detected and be acquainted by*:* Droping 1-2 drops of the Volatile Oil on a filter paper and then smelling.[15]

Specific Gravity
The specific gravity of volatile oil may be defined as the ratio of the weight of a given volume of the oil to the weight of the same volume of water, at a stated temperature. It is determined by the use of specific gravity bottle, Westphal balance or pycnometer.

Procedure
Add 3-4 drops of the above mentioned oils to a test tube containing 10ml. of water, shake gently set aside for few minutes and notice which one floats or sinks in water.

Solubility
The volatile oils are generally soluble in absolute alcohol, ether, ethyl acetate, chloroform, carbon disulfide, acetone, petroleum ether (Except cinnamic aldehyde and oils containing it) and benzene.[16]

Exp. Introduce 1ml. of the oil in 10 ml graduated cylinder and add alcohol of the specified strength gradually until complete solution occurs.

Determination of Saponification Value (SV)
To determine the Saponification Value (SV) of studied plant-oils, 0.5 gm of sample was dissolved in 12.5 ml of 0.5N Alcoholic KOH Solution. The mixture was incubated in boiling water bath for 30 minutes, which was then be cooled at room temperature, and titrated with 0.5N HCL with 1% Phenolphthalein indicator. Besides, a blank was also run to have precise comparison among duplicates, and the mean results were considered. The same protocol (Blank and Mean) was followed for all the other biochemical tests.
Saponification Value (SV) (mg KOH) = A x 28.06 / W
Where, A= Amount of HCL (ml) (Blank-Titer)
W = Weight of Sample (gm) Determination **of Iodine Value (IV)**

Gm of sample was dissolved in 10 ml of chloroform, to which 12.5 ml of Hanus Iodide Solution was added, and kept in dark for 30 minutes. Later, 15 ml of KI solution was added. The mixture was titrated with 0.1N Na2S2O3 Solution using 1% Starch Solution (Indicator).
Iodine Value (IV) = [(A x N x 0.1269 x 100) / W] gm I2 / 100 gm of Oil
Where, A = Amount of Na2S2O3 (Blank-Titer), N = Normality of Na2S2O3,
W = Weight of Sample (gm)

Determination of Acid Value (AV)
For determination of Acid Value (AV), 0.1 gm of sample was dissolved in 10 ml of Neutral Solvent. The mixture was titrated with 0.1N KOH Solution after an addition of few (2-3) drops of 1% Phenolphthalein Indicator.[17]
Acid Value (mg KOH / gm) = (A x 0.1 x 56.1) / W
Where, A = Titer Value (ml), 0.1 = Normality of KOH
W = Weight of Sample (gm)

Determination of Free Fatty Acids (%FA)
Percentage Free Fatty Acids (%FA) was determined using following equation:
%FA = K x Acid Value (AV)
Where, K = Constant (0.503).

Optical Rotation
The optical rotation and specific optical rotation give an indication whether the oil is genuine or adulterated. It may also indicate whether the substance is natural or synthetic.

The optical rotation is measured by the use of a polarimeter where a monochromatic sodium light and propagation tube containing the oil 10 cm (1 dm.) long is used. The measurements are carried at 25°c:

$$[\alpha]^{a}_{D} = a/C \times L$$

Refractive Index
The refractive index (nt) of volatile oils vary from 1.43-1.61.

It is commonly determined by the use of Abbe's refractometer.

Procedure
1. Weigh a sufficient quantity of the ground drug (fresh parts can be used) to yield if possible 2 ml. Of the volatile oil, and place it in the round bottomed flask.
2. Add from 3 to 6 times as much water as the drug and mix uniformly.
3. Boil the contents of the flask slowly during 4 to 8 hours, or until all the volatile oil has been distilled.
4. The volatile oil is collected in the apparatus according to whether it is heavier (A) or lighter (B) than water and its amount determined.[18]

FORMULATION OF TRANSDERMAL GEL
Preparation of herbal gel formulations
Carbopol® 940 gel base was prepared by hydrating Carbopol® 940 in propylene glycol and distilled water q.s. For 24 h followed by stirring with double bladed mixer at 200 rpm for 10 min. Then add herbal extracts like DMSO extract of *aloe Vera* gel, *lemongrass* oil and *coleus aromaticus* oil were subsequently mixed with carbopol gel base using spatula until homogenous stable herbal gels were formed. The gels were then transferred into clear glass vials.

Table No 1.2: Formula for polyherbal Transdermal gel.

S.No	Ingredients	Quantity Taken (%)
1	Carbapol	25%
2	Triethanolamine	0.5%
3	*Aloe Vera Gel*	0.5%
4	*Lemon Grass* Oil	1.2%
5	*Coleus Aromaticus* Oil	1.25%
6	Water	Q.S

PHYSICAL EVALUATION OF POLYHERBAL GEL

Percentage Moisture Content

Percentage moisture loss from the formulations was determined by (18) Two gram formulation was weighed accurately and kept in a desiccators containing 50gm anhydrous calcium chloride. After three days, the formulation was weighed. The percentage moisture loss was calculated using the formula as follows:

% moister content =initial weight-final weight/final weight×100

Transparency, smoothness and weight on drying

The 5ml gel formulation taken in the 10ml (Borosil^{R}) test tube and visually checked for its transparency. The smoothness of the gel formulation was tested by rubbing between the fingers and observes whether the gel is smooth, clumped, homogenous or rough. The relative density of the formulation or weight/ml of the formulation was determined by taking the weight in gm of 10ml formulation & 10ml distilled water using RD bottle.

Visual examination

All developed gel was inspected for their homogeneity (19), color; syneresis and presence of lumps by visual inspection after the gels have been set in the container.

Spreadability test

A sample of 0.5 g of gel was pressed between two slides (divided into squares of 5 mm sides) and left for about 5 minutes where no more spreading was expected.[20] Diameters of spreaded circles were measured in cm and were taken as comparative values for spreadability. The results obtained are average of three determinations.

P^{H} determination

The pH of the gel was determined using digital pH meter (21).The readings were taken for average of 3 times.

Drug Content determination

A specific quantity of developed gel was taken and dissolved in 100ml of phosphate buffer of pH 5.5. The volumetric flask containing gel solution was shaken for 2hr on mechanical shaker in order to get complete solubility of drug. This solution was filtered using Millipore filter (0.45µm). After suitable dilution drug absorbance was recorded by using UV- visible spectrophotometer (UV – 1700, Shimadzu, Japan) at λ_{max} 260 nm using phosphate buffer (pH5.5) as blank.

11. IN VITRO DRUG RELEASE

The study was carried out using (Varien dissolution tester, model VK 7010, with an auto sampler unit VK 8000, USA).One gram of gel was placed in the watch glass covered with aluminum mesh. The watch glass was then immersed in the vessel containing 500 ml of the release medium, phosphate buffer pH 5.5 at 37ºC ± 0.5 ºC with a paddle speed of 50 rpm. Aliquots (5ml) were withdrawn at specified time intervals every 10 minute over 2 hours and immediately replaced with fresh dissolution medium. The samples were assayed spectrophotometrically at λmax 260 nm and the concentration of the drug was determined from the previously constructed calibration curve. Experiments were carried out in triplicates, the results were averaged and blank experiments were carried using plain bases.[22]

THIN LAYER CHROMATOGRAPHY OF POLY HERBAL EXTRACTS

Thin Layer Chromatographic Characterization

Principle: The principle of separation is adsorption. One or more compounds are spotted on a thin layer of adsorbent coated on a chromatographic plate. The mobile phase solvent flows through because of capillary action (against gravitation force). The components move according to their affinities towards the adsorbent. The component with more affinity towards the stationary phase travels slower. The component with lesser affinity towards the stationary phase travels faster. Thus the components are separated on a thin layer chromatographic plate based on the affinity of components towards the stationary phase.

- **Stationary phase**

There are several adsorbents which can be used as stationary phases. Some of stationary phases used in TLC are Silica gel G, Silica gel GF, Alumina Cellulose powder, Kieselguhr G, Polyamide powder.

Silica gel G Composition : Silica gel +$CaSO_4$

Adsorbent water ratio : 1:2

- **Adsorbents**

Silica Gel (ACME Chemical works, Mumbai) was used for TLC.

Silica Gel mesh size 70-230 (Merck, Bombay) was used for column chromatography.

- **Glass plates**

Glass plates which are specific dimensions like 20cm X 20cm (full plate), 20cm X 10cm (half plate), 20 cm X 5cm (quarter plate) can be used. In general the glass plates should be of good quality and should with stand temperatures used for drying the plates.

- **Development tank**

For developing the chromatogram a developing tank or chamber of different sizes to hold TLC plates of standard dimensions are used. The development tank should be lined in side with filter paper moistened with mobile phase so as to saturate the atmosphere, this prevents edge effect.

- **Detecting or visualizing agents**

After the development of the TLC plates the spots should be visualized, coloured spots can be detected visually, but for detecting colorless spots any one of the following techniques can be used.

- **Nonspecific methods:** This is applicable where the no. of spots can be detected, but not the type of the compound.

Example

- Iodine chamber method
- Sulphuric acid spray reagent
- UV chamber for fluorescent compounds
- **Specific methods:** Specific spray reagents or detecting agents or visualizing agents are used for identification purpose

MATERIALS AND METHODS

Stationary phase-Silica gel G
Composition: silica gel +$CaSO_4$
Adsorbent: Water ratio - 1:2

PROCEDURE

Plate preparation: The slurry was prepared and poured on to the glass plate which was maintained on a level surface.

Activation of plate: The resultant plate was dried and activated by heating in an oven for 30min at 110^0C.

Mobile phase: Appropreate mobile phase is chosen for individual plant extracts. The solvents used for the plants were presented.

Development Tank: The prepared plates were developed in a TLC chamber. The development tank was lined in side with filter paper moistened with mobile phase so as to saturate the atmosphere, this prevents edge effect.

Development of plates and Derivatisation

TLC procedure was done under laboratory conditions of 29-33^0C and 50 % relative humidity. After development, the plate was removed and removed and dried and spots were visualized in UV light (254nm). The R_f values were calculated and presented.

The R_f Value is calculated using the formula:

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

IN VITRO ANTI FUNGAL EVALUATION OF POLYHERBAL EXTRACTS

Herbal extracts were tested for anti-fungal activity against test organisms namely *Candida albicans, Microsporum, Fusarium, Malassezia furfur* using modified agar well diffusion method [36]. In this method, nutrient agar plates and reinforced clostridial agar (RCA) were seeded with 100µl of standardized bacterial suspension [5x105 CFU/ml]. After optimization of dose, 200 mg of gel dispersion was poured into the wells. Standard Flucanozol (1% w/w) in-house gel (CLN) was used as positive control. The plates were then incubated at optimum temperature conditions and antibacterial activity was evaluated by measuring the diameter of zones of inhibition (mm) including cup size. The experiments were repeated three times.

RESULTS AND DISCUSSIONS

PHYTOCHEMICAL EVALUATION

Table No 1.3: Results for Phytochemical evaluation of aloe vera gel.

Chemical constituents	Results
Alkaloids	-
Flavonoids	-
Saponin	-
Resins	-
Sterols	+
Steroids & Terpenoids	-
Tannins	+
Glycosides	-
Acedic compounds	-
Carbohydrates	+
Reducing sugars	+
Anthrquinones	-

(+) = Present, (-) = Absent

PHYSICAL EVALUATION OF POLY HERBAL EXTRACTS

Table No 1.4: Results for physical evaluation of volatile oils.

Physical Parameter	Results	
	Lemongrass Oil	Coleus Aromaticus Oil
Saponification Value	200.44	180.60
Iodine Value	1.436	1.132
Acid Value	48.96	39.02
Free Fatty Acid Value	12.64	10.06
Opotical Rotation	-3 To +1.32	-3 To +1.5
Refractive index	1.433	1.44

PHYSICAL EVALUATION OF GEL

Table No 1.5: Results for Physical evaluation of gel.

Physical parameter	Results
% moister content	95.83
Transperency	Translucent
Smoothness/roughness	Smooth
Density	10.45
Spreadability(cm)	5
PH	6.9
Drug content	78.95

IN VITRO DRUG RELEASE

Table No 1.6: Results for *In-vitro* drug release.

Time	%Release
0	0
5	17.65
15	27.42
30	54.34
45	67.84
60	79.42

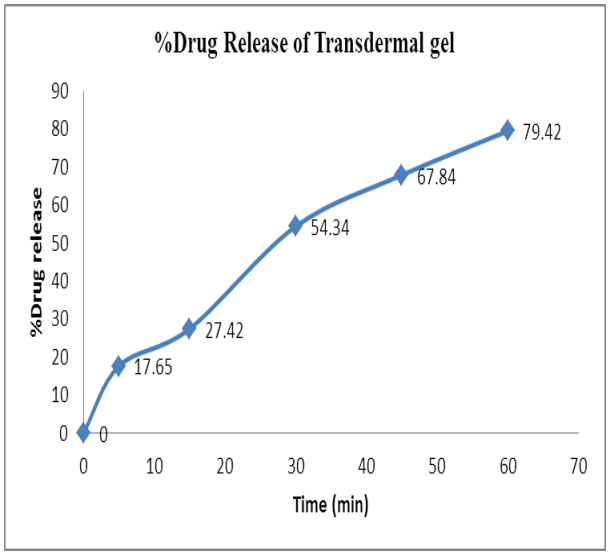
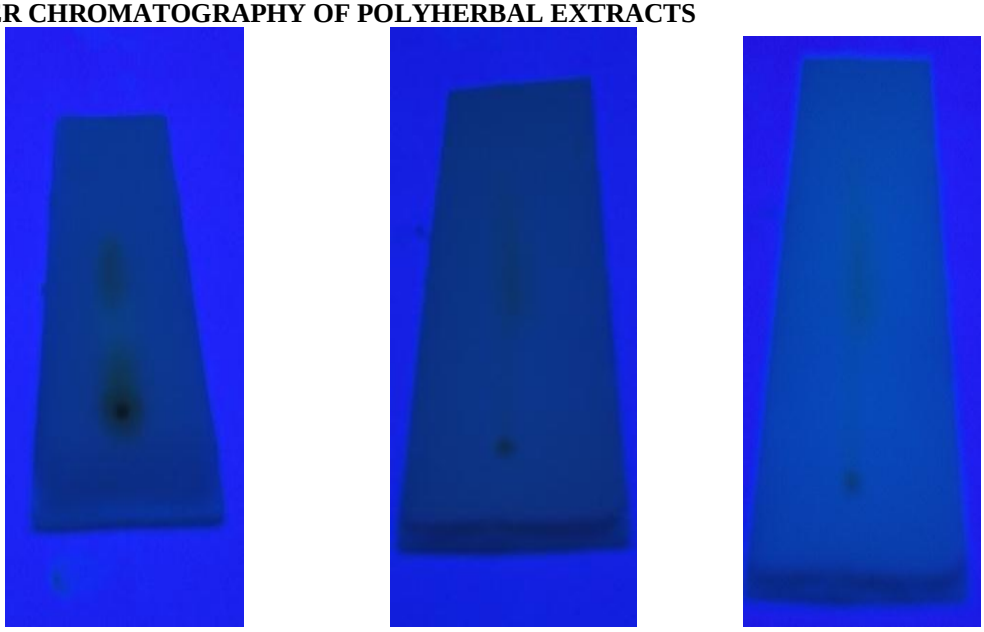


Figure No 1.1: Graphical representation for *In-vitro* drug release of Transdermal gel.

THIN LAYER CHROMATOGRAPHY OF POLYHERBAL EXTRACTS



TLC for *aloe vera gel* **TLC for *lemon grass oil*** **TLC for *coleus aromaticus***

Figure No 1.2: Thin Layer Chromatography Of *Polyherbal Extracts under UV-Lights*

Table No 1.7 Results for TLC of polyherbal extracts.

Sample	solvent	Under UV	
		R_f value	Colour
Aloe vera	Methanol :Ethyl acetate (3:7)	0.2896	Yellow
Lemon grass oil	Toluene :Ethyl acetate (7:3)	0.6875	Yellow
Coleusaromaticus oil	Hexane:Ehyl acetate:Acetia acid (6:3:1)	0.6864	Yellow

***IN VITRO* EVLUATION OF ANTI-FUNGAL ACTIVITY STUDIES**

1.3 images for *in vitro* evaluation of *lemon grass oil*

IMAGES FOR IN VITRO EVALUATION OF *COLEUS AROMATICUS* OIL

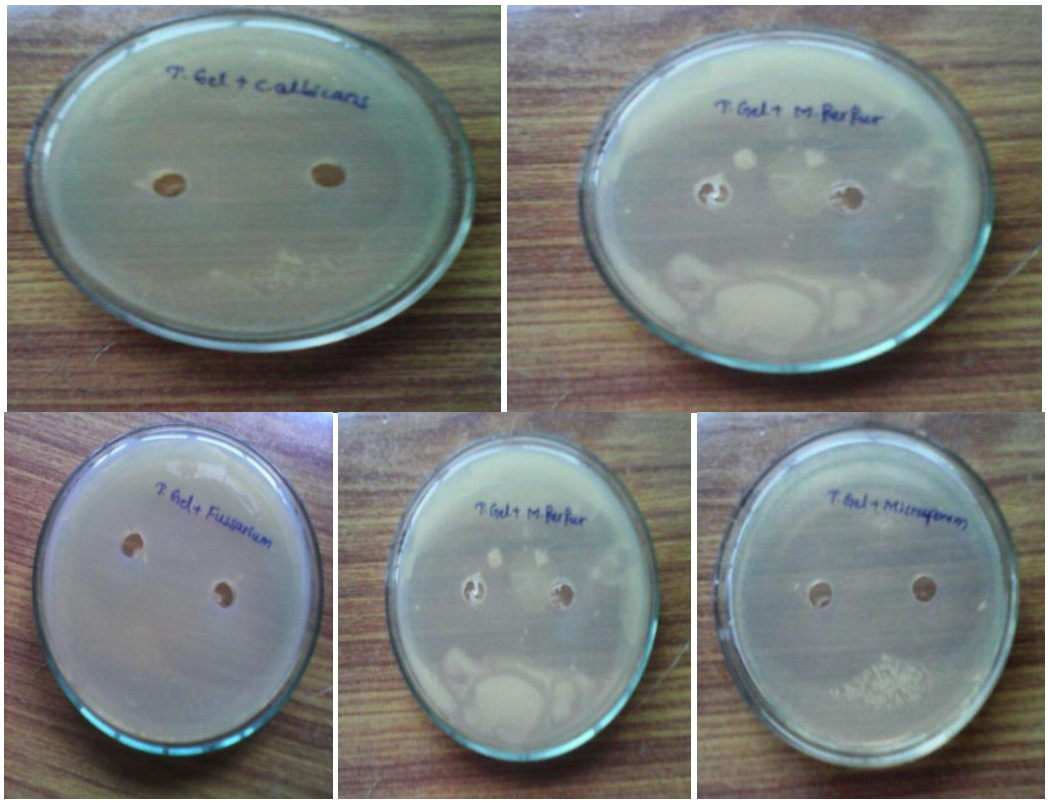
1.4 images for *in vitro* evaluation of *coleus aromaticus oil*

1.5 Images *for in vitro evaluation of aloe vera gel*

COMPARATIVE STUDIES

In vitro evaluation studies also carriedout for Formulation (Transdermal gel) and measured the Zone of inhibition in mm and compares the zone of inhibition of Transdermal Gel with the Individual polyherbal extracts. By doing comparision studies between the formulation and individual polyherbal extracts we can observe the maximum zone of inhibition for formulation (Transdermal gel).

IMAGES FOR *IN VITRO* EVALUATION OF TRANSDERMAL GEL



Images for in vitro evaluation of transdermal gel.

Table No 1.8: ***Results for In-vitro evaluation studies.***

Organisms	**Zone Of Inhibition(Mm)**			
	Aloe Vera	***Coleus Aromaticus* Oil**	***Lemon Grass* Oil**	**Transdermal Gel**
Candida Albicans	11.55	12.01	13.04	14.45
Microsporum	11.47	12.20	13.21	14.33
Fussarium	11.32	12.31	13.30	13.99
Malassezia Furfur	11.26	11.29	13.21	14.23

SUMMARY

In the present work, the pharmacognostical, phytochemical, Physical and In-vitro evaluation of oils of *Lemon grass, Coleua aromaticus* and DMSO extract of *Aloe vera* gel were carried out.

The results from phytochemical analysis indicated the presence of sterols, tannins, carbohydrates and reducing sugars.

Phytochemical screening, TLC analysis was performed in order to determine the chemo profiles. TLC studies were carried out for *Lemon grass* oil. *Coleus aromaticus* oil, and *aloe vera* gel. The suitable solvent system for plant extracts was established and the numbers of spots were determined. The R_f values of the spots were recorded. Results from TLC also confirmed the presence of phytochemicals in the poly herbal formulations.

The plant extracts of *lemon grass* oil, *Coleus aromaticus* oil, and DMSO extract of *Aloe vera* gel were found to be Anti fungal. Anti fungal activity was carried out by *In-vitro* disc diffusion method. The poly herbal extracts had showed a significant anti-fungal activity against the fungal species like *Candida albicans, Microsporum, Malassezia furfur, Fussarium.* The polyherbal extracts showed a significant anti fungal activity.

Summarizing the work, it was clearly obvious that the polyherbal extracts have shown a noteworthy achievement as an anti-fungal activity.

In India more than 70% of the population uses herbal drugs for their health. There is a vast experience-based evidence for many of these drugs. There are also a number of Institutes/Universities in India carrying our research on herbal drugs and medicinal plants. The after effects on using allopathic medicines added immense pressure for theinvent of herbal medicines. The urbanization and life styles of people made them depend on immediate responsive commercial drugs neglecting their side effects. But, long term usage and deterioration of conditions fortunately shifted the world for an alternative medicine.

CONCLUSION

The present work was an attempt to evaluate polyherbal extracts for anti-fungal activity. Results obtained from the present study provided evidence that polyherbal extracts possessed an anti-fungal activity. In-vitro anti fungal evaluation studies were carriedout for both plant extracts and formulation. In the present study it was demonstrated that external administration of polyherbal extracts shows significantly anti fungal activity.

Comparision studies were carriedout between the polyherbal extracts and Transdermal gel. Maximum zone of inhibition was observed for formulation as compared to individual plant extracts.

Based on the present study, it can be concluded that polyherbal extracts possesses anti-fungal activitiy. But by doing comparision studies maximum zone of inhibition was observed for formulation (Transdermal gel) compared to polyherbal extracts.

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