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FORMULATION AND IN VITRO – IN VIVO EVALUATION OF CONVOLVULUS PLURICAULISCUBOSOMES FOR BRAIN TARGETING TO ENHANCE THE MEMORY

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Abstract: It was determined that the findings of this study have the potential to contribute to the treatment of psoriasis since the only various therapies that are presently available aim solely to lessen the intensity of the symptoms. Therefore, there is a need for treatments that are efficient, inexpensive, and have fewer side effects that target the aetiology of the condition. Both ethyl acetate and metabolic extracts of herbs are exhibiting considerable anti-psoriatic efficacy via the reduction of keratinocyte growth.

Introduction

The plants were identified and collected from Tirupati hills, Andhra Pradesh and authenticated by the botanist for the correct identification of plants. The medicinal value of a crude drug depends on the presence of chemical constituents of physiological importance. The compounds that are responsible for the therapeutic effect are usually the secondary metabolites. All the collected plants are cleaned from debris washed, shade dried and were coarsely powdered and mixed in equal ratio and extracted using different organic solvents like of hexane, chloroform, ethyl acetate and methanol based on polarity. The medicinal value of a crude drug depends on the presence of chemical constituents of physiological importance. The compounds that are responsible for the therapeutic effect are usually the secondary metabolites. So the plant materials are subjected to preliminary screening for the detection of various plant constituents. The solvent is removed from extracts by distillation under reduced pressure. The concentrated extracts were kept in a dessicator and were used for further experiment. Each extract was weighed and its percentage in terms of air-dried weight of plant material was calculated and also the consistency of the extracts was noted. The hexane, chloroform, ethyl acetate and methanol extracts of the plant extracts were subjected to identification of phytoconstituents. Total flavanoid and alkaloids content in Plant extract was estimated by spectrometric method. Thin layer chromatographic studies (TLC) were done. HPTLC technique is useful for identification of plants and their extracts because each plant species produce a distinct chromatogram with unique marker compounds used for plant identification. In cytokine inhibition assay, the alkaloidal and flavonoidal fraction showed remarkable inhibition of IL-17 and TNF- α, key cytokines involved in the pathogenesis of psoriasis at higher concentration. The serum TNF-α, IL-1, IL-6, IL-8, IL-12, and IL-17 levels were significantly higher in active psoriatic patients than in controls. Regulation of the inflammatory events initiated or perpetuated by keratinocytes could so represent an important strategy for the treatment of psoriasis and other chronic inflammatory skin diseases.17

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Thus inhibition of TNF-α, IL-1, IL-6, IL-8, IL-12, and IL-17 could be employed as criteria for the evaluation of anti-psoriatic activity.

From the *in-vivo* in vitro studies it was concluded that the ethyl acetate and methanolic extract of herbs cassia sophera and mallotus philippinensis showed significant anti psoriatic activity and it will be a promising alternative treatment for diseased population.

Literature Survey

Herbal remedies have been highly valued ever since ancient times for its analgesic and therapeutic characteristics. Even in modern times, we continue to place a significant amount of importance on the medicinal qualities of plants. Over the course of many generations, cultures all over the globe have independently established their own traditions in order to make sense of medicinal plants and the applications for those plants. It should not come as a surprise that the regulatory authorities have only authorised a limited number of medications for the treatment of multi-factor illnesses like Alzheimer's disease since the brain is one of the body's most complicated organs.

Evolvulus alsinoides is one of the most well-known and significant plants used for medicinal purposes in India. This plant is also known to have a variety of beneficial effects on human health. All portions of Evolvulus alsinoides exhibit excellent therapeutic characteristics. The practise of ayurveda makes extensive use of this herb. Therefore, in this research, has been taken and analysed for its effectiveness against reserpine-induced orofacial dyskinesia.

The plant that has been found to include Shankapushpine and Betaine also has volatile oils and potassium chloride within its composition. In addition to these, it has a yellow neutral fat, an organic acid, and salty components, and its extract shows a positive result for sterols and alkaloids while demonstrating a lack of phenols, tannins, carbohydrates, and proteins.

Since ancient times, people have placed a significant amount of importance on herbal treatments due to the analgesic and therapeutic properties that they provide. Even in our contemporary times, we continue to put a large degree of weight on the fact that different plants have different properties that might be used for therapeutic purposes. In order to make sense of medicinal plants and the uses for those plants, nations all over the world have separately developed their own traditions over the course of many centuries. These traditions have been passed down from generation to generation. Since the brain is one of the most complicated organs in the body, it should not come as a surprise that the regulatory authorities have only authorised a limited number of medications for the treatment of multi-factor illnesses like Alzheimer's disease. This should not come as a surprise because it should come as no surprise at all.

One of the most well-known and prominent plants that is utilised for medicinal reasons in India is a species of Evolvulus called Evolvulus alsinoides. It is well recognised that this plant is good to both human and animal health in a number of different ways. Excellent therapeutic properties may be found in every part of the Evolvulus alsinoides plant. This plant is used rather extensively throughout the practise of ayurveda. Therefore, in the course of this investigation, has been obtained and examined for its efficiency in combating the orofacial dyskinesia that is caused by reserpine.

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In addition to shankapushpine and betaine, researchers discovered that the plant also included volatile oils and potassium chloride in its make-up. In addition to these, it has a yellow neutral fat, an organic acid, and salty components, and its extract shows a positive result for sterols and alkaloids while exhibiting a lack of phenols, tannins, carbohydrates, and proteins. These components may be found in the plant's extract.

Objective of the project

The objectives of this research were:

- To separate steroid fraction from Indian medicinal plants
- To standardize the fractions for phytoconstituents using TLC, HPTLC and HPLC.
- To study the effect of the fractions on the level of cytokine-IL-17, IL-22, TNF-γ, lipooxygenase and keratinocytes.
- To study the anti-psoriatic activity using in-vivo models.

Methodology

i. Selection of Indian medicinal plants

The Indian medicinal plants [Cassia sophera (Family: Fabaceae), Mallotus philippinensis (Family: Euphorbiaceae)] traditionally used for treatment of Psoriasis was collected from Tirumala Hills, Tirupati, Andhra Pradesh for the present study. The plant material was identified and authenticated by Botanist.

ii. Collection and Authentication of selected plants:

The Indian medicinal plants [Cassia sophera (Family: Fabaceae), Mallotus philippinensis (Family: Euphorbiaceae)] are collected from Tirupati hills and also from Chittoor, Andhra Pradesh, and authenticated Botanist Dr. P. Jayaraman, Director, Plant Anatomy Research Centre (PARC), Tambaram, Chennai, Tamil Nadu, A voucher specimen (SVCOP- 1-2016) of the plants no: SVCOP 2016/025 and 026 has been deposited at the herbarium unit of the Department of Pharmacognosy, Sri Venkateswara College of Pharmacy, Chittoor.

iii. Preparation of Extracts

All the plants were washed and air dried individually and after drying the plant material is individually powdered coarsely. The Coarsely powdered dried aerial part of Cassia sophera and Mallotus philippinensis were mixed in equal ratio 1:1:1 (5 Kg) were extracted in 50 % aq. Ethanol and the extract is fractioned with various solvents like hexane, chloroform, ethyl acetate and methanol to yield the respective fractions. All the fractions were collected in a 5 liter conical flask, filtered, and the solvent was evaporated to dryness under reduced pressure in a Rotary evaporator at 40^{o}-45^{o}C. All the fractions were stored in a well closed air tight container and kept in desiccators and it is used for preliminary phytochemical analysis.

iv. Preliminary Phytochemical analysis of different extracts of herbs

The preliminary phytochemical group tests of various extracts of Plants were performed by the standard methods (Kokate. 2005) to identify the presence of various chemical constituents.

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IN-VIVO ANTIPSORIATIC ACTIVITY

Animals

Healthy male Wistar rats (120-170 g) and Swiss albino mice (25- 30 g) obtained from the institutional animal housing facilities were used for the study. Animals were housed in polypropylene cages and were left seven days for acclimatization to animal room, which was kept under controlled conditions (a 12 h light-dark cycle at 22±2°C) and fed on standard pellet diet and water ad libitum. All animals were taken care of under ethical consideration as per the guidelines of CPCSEA with approval from the Institutional Animal Ethics Committee ([CPCSEA] IAEC/SVCOP /52/2017).

Acute toxicity studies

During the acute toxicity study, th methanolic and ethylacetate extract, was administered orally and animals were observed for mortality and behavioral responses. There was no mortality observed even at 2000 mg/kg for the extract and at 500 mg/kg for the compounds. All the animals were normal and there were no gross behavioral changes till the end of the observation period.

Rat ultraviolet ray B photo dermatitis model for psoriasis

Mice (six animals per group) were exposure of the rat's skin to UV radiation using a UV-B bulb (wavelength 280-315 nm) induced proinflammatory reaction in the skin that resembles the one observed in psoriasis. This was evident by the altered skin parameters; the most important of which are the increase in epidermal thickness to almost double the normal size, absence of stratum granulosum and the movement of neutrophils towards the epidermis, all symptoms typical of psoriasis., (Michael et al., 2005).

The hairs of one side of the flank of the rat were clipped with scissors followed by careful shaving, taking precaution to avoid injury to the skin. The animals were then placed on a curved wooden block and their legs tied around it, to avoid contact with the floor. This arrangement prevented the movement of the animal during its subsequent exposure to UV radiation. into seven groups (six animals per group). The control group animals received normal Except for an area of 1.5 × 2.5 cm on the depilated skin, the entire animal was covered with a UV resistant film. The uncovered area of 1.5 × 2.5 cm was then irradiated for 20 min with a UV-B lamp kept at a vertical distance of 20 cm from the skin (Vogel et al., 2002). The animals were divided saline (10 ml/kg, p.o.) and standard group received retinoic acid (0.5 mg/kg, p.o.). The remaining groups were treated orally with the methanolic and ethyl acetate extract of plant herbs (200 and 400 mg/kg)) once daily, five times a week, 12 h after irradiation, for two weeks. Two hours after the last treatment animals were sacrificed; longitudinal sections of the tail skin were made and prepared for histological examination with hematoxylin-eosin staining (Nakaguma et al., 1995; Singhal and Kansara 2012). Histopathological examination Sections were examined for the presence of Munro's microabscesss, elongation of rete ridges, and capillary loop dilation by direct microscopy. The vertical epidermal thickness between the dermoepidermal junction and the lowest part of the stratum corneum (n = 3 measurements per scale, n = 3 scales per animal, n = 6) were examined. The percentage relative epidermal thickness of all the groups was calculated in comparison to the positive control group (100%; n = 54 measurements per treatment). It was also examined for Mean thickness of stratum corneum and stratum granulosum. All

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measurements were made at a magnification of 400× using a digital camera attached to an Olympus microscope, and used software to take measurements.

Statistical analysis

Values were represented as mean ± SEM. Data were analyzed using one-way analysis of variance (ANOVA), and group means were compared by the Tukey-Kramer Multiple Comparison test using Instat-V3 software. p values < 0.05 were considered significant.

Results and Discussions

Table 1: Ash values of*POLYHREBS*

Total ash (%)	Acid Insoluble ash (%)	Water soluble ash (%)
11.23±.115	1.126±0.34	13.340±1.45

Table 2. moisture and fat content of polyherbs

Parameter	%
1. Moisture content	5.4
2. Fat content	2

Table 3. Biochemical composition of polyherbs

Parameter	Values
Carbohydrate mg/g	0.462±0.012mg/g
Protein mg/g	13.29±0.244 mg/g
Amino Acid mg/g	2.224±0.160 mg/g
Total free Phenols (mg/g)	14.3±0.126 mg/g
Tannins (mg/g)	12.1±0.27mg/g
Total Flavonoids **Total alkaloids**	**91.09± 0.04mg of QEof extract in ethylacetate fraction** **66.08± 0. 33mg/g in methanolic fraction** **66.01± 0.049 mg/g AE/mg of** **91.09± 0.04mg/g**

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Table 4: Estimation of metals/Heavy metals in dried material of *Polyherbs*

S.No	Name of the metal/Heavy metal	Amount in mg
1.	**Sodium**	57.34
2.	**Potassium**	49.32
3.	**Phosphorous**	23.6
4.	**Magnesium**	0.89
5.	**Calcium**	67.7
6.	**Copper**	0.12
7.	**Selenium**	0.6
8.	**Zinc**	0.32
9.	**Lead**	Less than 1 PPM

Table 5: Rf values of standard amino acids and sample of *Polyhrebs* by *THIN LAYER CHROMATOGRAPHY*

S.No	Name of the Amino Acid	Rf value	S.No	Name of the Amino Acid	Rf value
1.	L- Proline	0.379	**14.**	L- Histidine	0.102
2.	L-Serine	0.214	**15.**	DL- 2-amino-N-butric acid	0.355
3.	DL-Nor leucine	0.695	**16.**	L.Glycine	0.193
4.	L.Ornithine	0.129	**17.**	L.Arginine	0.163
5.	DL.Threonine	0.238	**18.**	L- Tyrosine	0.373
6.	L-Cysteine	0.121	**19.**	Phenyl alanine	0.587
7.	L-Leucine	0.677	**20.**	Lysine	0.141
8.	DL-Valine	0.468	**21.**	Tryptophan	0.627

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9.	3-3,4-dihydroxyphenyl alanine	0.280	**22.**	Methionine	0.156
10.	L.Hydroxy proline	0.218	**23.**	L- Cysteine	0.419
11.	DL- Isoleucine	0.618	**24.**	L – Aspartic acid	0.349
12.	L. Alanine	0.227	**25.**	SAMPLE	0.162,0.236 0.226,0.158 0.162
13.	L- Glutamic acid	0.160			

Table 6: Percentage yield of total extract of plant material

Parameter	70% Ethanolic extract
Colour of extract	**Dark green**
Consistency	**Semisolid**
Percentage yield (% w/w)	**12.50**

Table 7: Preliminary Phytochemical Analysis of various fractions of *Polyherbs* (Kokate,1997

Chemical Test	Hexane fraction	Chloroform fraction	Ethyl acetate fraction	Methanol fraction
Alkaloids	-	-	+	+
Carbohydrates	-	-	-	+
Steroids	+	+	+	+
Tannins	-	-	-	+
Proteins	-	-	+	+
Terpenoids	-	-	-	-

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Flavonoids	-	-	+	+
Gums &mucilage	-	-	-	+
Oils &fats	+	+	+	+

It is observed that preliminary phytochemical studies show the presence of flavanoids, alkaloids, terpenoids, tannins, amino acids, carbohydrates and steroids.
The preliminary phytochemical screening of ethyl acetate and methanol fraction of *Cassia sophera* and *Mallotus philippinensis*was performed. The Preliminary phytochemical analysis was made clearly indicated the presence of alkaloids, proteins, glucosides, steroids, tannins and flavonoids.

Determination of Steroids
The highest concentration of steroids was measured 24.44± 0.024mg of extract in ethyl acetate fraction and 37.29± 0.41mg of AE/g of extract in methanolic fraction.
Determination of total alkaloid contents
The alkaloid contents were examined in plant extracts and expressed in terms of atropine equivalent as mg of AE/g of extract. The highest concentration of alkaloid was measured 66.01± 0.049mg of extract in ethyl acetate fraction and 41.08± 0.33mg of AE/g of extract in methanolic fraction.

Determination of total flavonoid content
The content of flavonoids was expressed in terms of Quercetin equivalent mg of mg Quercetin/g of extract. The concentration of flavonoids in ethyl acetate and methanol fraction of *Cassia sophera* and*Mallotusphilippinensis* was estimated 91.09± 0.04mg/g and 66.08± 0.33mg/g. High solubility of phenols and flavonoids in polar solvents provides high concentration of these compounds in the extracts obtained using polar solvents for the extraction.

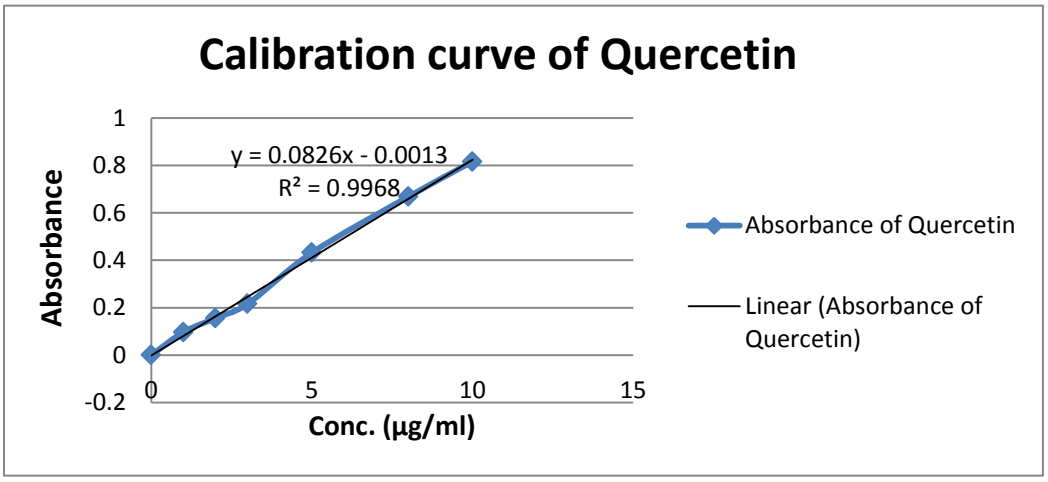

Figure 1. Calibration curve of Quercetin

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Table 8: Protein as Amino Acids was determined by using HPLC System (LACHROM – 700) in extracts

S.No.	Name of the Amino Acid	Amount in mg
1.	Glutamic acid	**0.089**
2.	Asparagine	**0.056**
3.	Glutamine	**0.034**
4.	Arginine	**0.317**
5.	Alanine	**0.125**
6.	Threonine	**0.206**

Table 9 : R_f values of ethyl acetate and methanolic fractions of Polyherb by HPTLC

S. No.	Name of the fraction	Solvent system	Detection wavelength	No. of spots	R_f value
1	Ethyl acetate	chloroform: methanol: formic acid: glacial acetic acid (7:2:1: 1)	254 & 366	09	0.07, 0.13, 0.18, 0.31, 0.42, 0.54, 0.64, 0.72, 0.88
2	Methanol	ethyl acetate: hexane (4:6)	254	10	0.04, 0.07, 0.16, 0.24, 0.37, 0.51, 0.57, 0.65, 0.76, 0.82

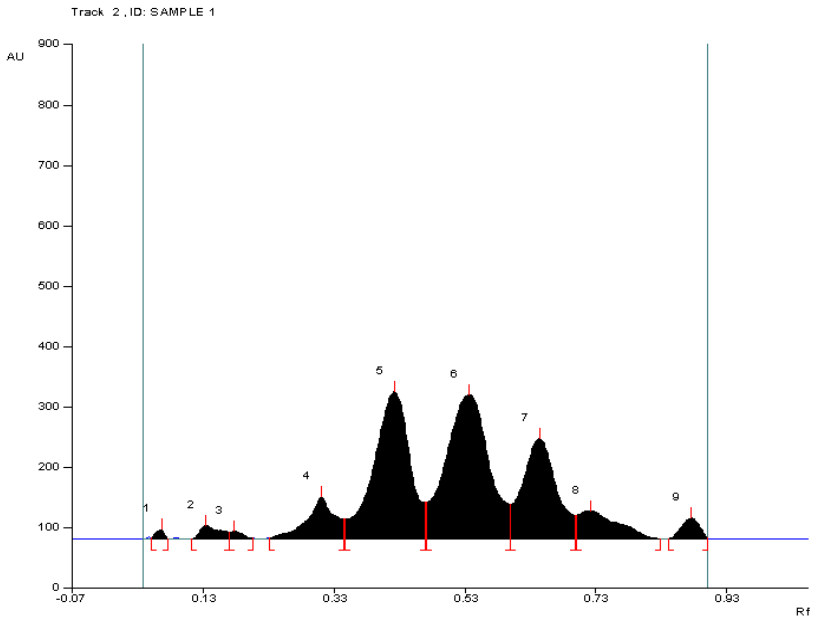

Figure 2 : HPTLC profile of ethyl acetate fraction

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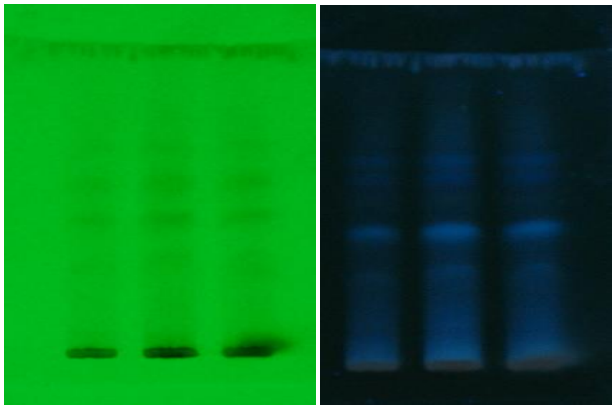

254 nm **366 nm**

Figure .3: TLC Profile of ethyl acetate fraction

HaCaT keratinocytes cell inhibition assay

The cytotoxic effect of ethyl acetate and methanol fraction of *Cassia sophera* and *Mallotusphilippinensis* were evaluated using HaCaT cells, a rapidly multiplying human keratinocyte cell line, as a model of epidermal hyper proliferation in psoriasis. The tested extract showed appreciable antiproliferant activity in HaCaT cell line due to the presence of flavonoids. The results were validated using asiaticoside as positive control. Ethyl acetate fraction of *Cassia sophera* and *Mallotus philippinensis* showed antiproliferant activity significantly (24.27±4.94µg/ml)in HaCaT cell line. Methanol fraction of *Cassia sophera* and *Mallotusphilippinens is* showed appreciable antiproliferant activity (29.27±5.67µg/ml) in HaCaT cell line. Asiaticoside showed a potent activity with IC50 value of 33.69 µg/ml.

The cytotoxic effect of ethyl acetate and methanolic fraction were evaluated using HaCaT cells, a rapidly multiplying human keratinocyte cell line, as a model of epidermal hyperproliferation in psoriasis. Both the fractions showed significant antiproliferant activity in HaCaT cells. The results were validated using asiaticoside as positive control and tabulated. In cytokinin inhibitory assay showed Inhibitory effects of the test samples on IL-lα, IL-1β, IL-6, IL-8, IL-17, TNF-α biosynthesis as the inhibitory percentages. For 70 and 100% is accepted as high, values between 40 and 69% as moderate, 20 and the interpretation of the results, percentage values are classified under four groups; an inhibition between 39% as low and an inhibition less than 20% is considered to be insignificant

Psoriasis can be described as a T-cell-mediated disease, with a complex role for a variety of cytokines and other factors. Interaction between T lymphocytes and keratinocytes, via cytokines, is likely to play a pivotal role in the pathogenic process in psoriasis. The Th1 cytokines (TNF-α, IFN-γ, and IL-12) and some proinflammatory cytokines (such as IL-6, IL-8, and IL-18) are influenced in the serum of psoriatic patients.

Th17 cells are stimulated by IL-23 (which shares the p40 subunit with IL-12) to produce IL-17 and also IL-22, which has recently been shown to be a major driver of acanthosis in psoriasis, and so is a novel target for treatment.17 The exact role of TNF-α in the pathomechanism of psoriasis is still unclear, but anti-TNF-α therapy is highly effective in psoriasis indicating that this cytokine has, together with IFN-γ, a central role in the pathogenesis. IFN-γ and TNF-α

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induce IL-6, IL-8, IL-12, and IL-18 and constitute an important link in the cytokine network in the pathogenesis of psoriasis.

Conclusion

It was concluded that this research can be contributed to treat the psoriasis because only multiple treatments are currently available only to reduce the severity of the symptoms. So there is a need for effective ,affordable therapies with fewer side effects that address the cause of disorder and the ethyl acetate and metabolic extract of herbs are having significant anti psoriatic activity through the inhibition of keratinocyte proliferation.

The following research works are to be carried out in future (future work)

- Scaling up of methodology to industrial scale by making creams, ointments and capsules
- To conduct clinical trials in order to prove the quality, safety and efficacy of dosage form.
- To identify which constituents responsible for anti psoriatic activity

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