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Original Article

Physicochemical and *in vitro* Analysis of Herbal Drugs *A. indica, C. longa, P. pinnata, P. corylifolia, W. fruticosa* for Potential Effect in Psoriasis

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ABSTRACT

Background: *Curcuma longa* (Zingiberaceae), *Azadirachta indica* (Meliaceae), *Psoralea corylifolia* (Fabaceae), *Pongamia pinnata* (Fabaceae), and *Woodfordia fruticosa* (Lythraceae) stand as renowned medicinal plants in India. Traditionally, different parts of these plants have been employed for healing, particularly in the context of treating psoriasis and various other ailments. This catalyzed our investigation into the potential antipsoriatic properties of these botanicals. **Objectives:** We set out to evaluate the preliminary phytochemical analysis and *in vitro* antipsoriatic capabilities of acetone, ethanol, and water extracts derived from *Curcuma longa, Azadirachta indica, Psoralea corylifolia, Pongamia pinnata,* and *Woodfordia fruticosa*. **Materials and Methods:** The antipsoriatic potential of these extracts was assessed through the MTT assay, using HaCaT cells. Additionally, we conducted LDH assays and utilized confocal microscopy to gain further insights. **Results:** Our investigations unveiled the promising antiproliferative activity of these plants on skin keratinocytes. Notably, compounds A, B, and C demonstrated a significant decrease in cell viability compared to the control group. In contrast, compounds D and E did not exhibit significant differences compared to the control. **Discussion and Conclusion:** These findings substantiate the traditional uses of these plants in the treatment of psoriasis and underscore their potential as valuable resources in the quest for effective antipsoriatic treatments.

Keywords: *Curcuma longa*, *Azadirachta indica*, *Psoralea corylifolia*, *Pongamia pinnata*, *Woodfordia fruticosa*, haCaT cells.

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INTRODUCTION

Psoriasis, an ailment affecting the skin, manifests when the body's immune system sends erroneous signals, leading to an accelerated skin cell growth cycle. This condition is a non communicable, multifaceted autoimmune skin disorder, recognizable by the presence of reddish, silvery patches on the skin, excessive keratinocyte proliferation, heightened dermal vascularity, and persistent inflammation.[1,2] This malady has been known for centuries, and presently, approximately 2-3% of the global population grapples with it. Psoriasis carries significant social, psychological, and economic implications.[3] Its impact on quality of life closely resembles that experienced by individuals coping with other chronic health conditions, such as diabetes and depression.[4,5]

Numerous scientific studies have provided evidence those certain medicinal plants, such as Radix pae, *Rubia cordifolia* Linn. (Rubiaceae), *Coptis chinensis* Franch. (Ranunculaceae), *Alpinia galangal* Linn. (Zingiberaceae), *Annona squamosa* Linn. (Annonaceae), *Curcuma longa* Linn. (Zingiberaceae), and others, exhibit effectiveness in the treatment of psoriasis.[6-12]

In this research, we focused on five Indian medicinal plants, namely *Curcuma longa* (Zingiberaceae) *Azadirachta indica* (Meliaceae) *Psoralea corylifolia* (Fabaceae) *Pongamia pinnata* (Fabaceae) and *Woodfordia fruticosa* (Lythraceae) to assess their impact on skin keratinocyte proliferation. These plants have a well-established history of use by traditional healers for the treatment of chronic skin inflammation and conditions resembling psoriasis. Despite the historical usage of these plants, there is a scarcity of scientific research examining their anti-psoriatic potential. The primary objective of this study was to uncover the therapeutic benefits these medicinal plants may offer in the context of psoriasis treatment. To achieve this, we employed a cultured HaCaT cell line, which is a well-established *in vitro* model for assessing the antipsoriatic properties of novel treatments, owing to its highly preserved differentiation capacity.

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MATERIALS AND METHODS

Chemicals and Reagents

MTT, and DMSO were purchased from Sigma-Aldrich. Amphotericin B antibiotic and Cell culture media were supplied by Gibco. Gentamicin, FBS (fetal bovine serum) was obtained from thermos fisher. Primary antibodies (Caspase 7) were purchased from Elabsciences. Secondary antibodies (Alxa flouro @488) were purchased from sigma Aldrich.

Plant Material

All the plants used in this study were obtained as a gift sample from Dhanwantri Herbals Amritsar, Punjab, India. The plant's part was washed, shade dried and powdered. The specimen was authenticated by submitting it to the Shree Guru Nanak Dev University Amritsar and voucher no. is 490 Bot. and Env. Sc.

Physicochemical Evaluation

The physicochemical parameters were evaluated to ensure the quality of raw herb, which has to be used further for extraction purpose. It includes foreign matter, loss on drying, total ash, acid insoluble ash, WSEV, ASEV, heavy metal analysis, lead, cadmium, mercury and arsenic (Tables 1-6).[13]

Extraction

Soxhlet Extraction

Take 4 g of coarsely powdered drug and placed in filter paper (Whatman No.1) thimble in soxhlet apparatus fitted with 250 mL of round bottom flask containing 150 mL of solvent. Extraction was performed at boiling temperature of respective solvent for 5 hr with completion of up to seven cycles through siphon mechanism in case of extraction with acetone and ethanol. In case of extraction with water, time required for completion of one cycle was significantly more hence, with water extraction was carried out for longer time with the completion of up to seven to eight cycle through siphon mechanism. After the completion of first extraction step, residue in the thimble was again extracted twice with suitable amount of respective solvents. Filtrate of each solvent from three extraction steps were taken out and their volumes were noted.

Qualitative Phytochemical Estimation of Extracts

Phytochemical screening was performed on the plant's extracts (obtained in solvents such as Ethanol, Acetone, and water) to determine the presence of phytochemicals such as carbohydrates, alkaloids, flavonoids, glycosides, proteins and amino acids, saponins, triterpenoids and steroids, tannins and other phenolic compounds. [Table 6] These plant extracts were obtained using soxhlet extraction techniques. To identify the constituents in the different plant extracts, specific qualitative phytochemical tests were performed.[14]

In vitro Assay

Preparation of test materials for bioassay

Cell Culture

The keratinocyte cell line HaCaT was obtained from NCCS, Pune Bangalore. Chemically defined Serum-free KGM were provided by Gibco Co. (North Andover, MA, USA). The HaCat cells were maintained in KGM medium at 37°C, 5% CO_2 until 70% confluency.

MTT assay

The cells were treated with positive control (PMA), compound A, B, C,D and E for 24 hr. The Compound A,B,C,D and E were evaluated for cytotoxic activity against HaCat keratinocyte cell line. The cells were plated separately in 96 well plates at a cell density of 5000 cells/well and incubated at 37°C, 5% CO_2 until 70% confluency. After 48 hr, cells were washed twice with 100 µL of Phosphate Buffer saline. After washing, cells were treated with the test material (Compound A, B,C,D and E) at concentration of 10 ug/mL for 24 hr. At the end of the 24 hr after treatment period, the medium was aspirated. The MTT was prepared in PBS (pH=7.2) at concentration of 5mg/mL. The serum free medium KGM containing 20 uL of MTT (5 mg/mL) was added and incubated for 3 hr at 37°C in a CO_2 incubator. The MTT containing medium was then discarded and the cells were washed with PBS (200 µL). The cells were then incubated with 100 µL of DMSO for dissolving crystals. This was mixed properly by pipetting up and down. Spectrophotometric absorbance of the purple blue formazan dye was measured in a microplate reader at 570 nm (Biorad 680). The experiment was done three times in triplicates and the average of the viability was calculated.[15]

ELISA technique

The cells were treated with positive control (PMA), compound A,B,C,D and E for 24 hr and after incubation the ELISA experiment was conducted as per manufacturer kit instructions. This sandwich kit is for the accurate quantitative detection of human IL-10,IL12 and IL-13 cytokines. These kits are solid-phase sandwich ELISA. The 96 well plates have been pre-coated with human antibody of IL-10,IL-12 and IL-13 cytokines. All the reagents, standard solutions and samples were prepared as per instruction manual. All reagents were brought to room temperature before use. The assay was performed at room temperature. 50 µL of standard were added to standard well. 40 µL of cells homogenate sample were added to sample wells and then add 10 µL of antibody IL-10,IL-12 and IL-13 cytokines in their respective wells were added to sample wells and after that 50 µL streptavidin-HRP were added to sample wells and standard wells (Not blank control well).All the reaction mixture were mixed well. the plate was covered with a sealer. The plate was incubated for 60 min at 37°C.Ater incubation, plate was washed for 5 times with the wash buffer, which was provided with the kit.

The wells were soaked with at least 0.35 mL wash buffer for 30 sec to 1 min for each wash. After washing, 50 µL of substrate solution A was added to each well and then 50 µL substrate solution B were added to each well. The plate was incubated for 10 min at 37°C in the dark. After incubation, 50 µL of stop Solution were added to each well and the blue color would change into yellow immediately. The optical density of each well was determined immediately using a micro plate reader at 450 nm wavelength within 10 min after adding the stop solution.[16]

LDH Assay

LDH is an oxidoreductase enzyme that catalyses the inter conversion of pyruvate and lactate. Cells release LDH into the bloodstream after tissue damage or red blood cell hemolysis. Since LDH is a fairly stable enzyme, it has been widely used to evaluate the presence of damage and toxicity of tissue and cells. Quantification of LDH has a broad range of applications. LDH Activity Assay Kit has been used for measuring the activity of LDH in the cell culture medium. We have used LDH Activity Assay Kit (MAK066, Sigma Aldrich) according to manufactures instructions. In this kit, LDH reduces NAD to NADH, which was specifically detected by colorimetric (450 nm) assay. The LDH Activity Assay kit has quantifies LDH activity in cells of treated and control group. All the vials were briefly centrifuged before opening. The ultrapure water was used for the preparation of reagents. LDH Assay Buffer was allowed to come to room temperature before use. LDH Substrate Mix were reconstituted in 1 mL of water. All the reagents were mixed well by pipetting and kept at cold while in use. Substrate Mix was stable for one week at 4ºC and 1 month at -20°C. 1.25 mM NADH Standard were reconstituted in 400 L of water to generate 1.25 mM standard solution. All the reagents were mixed well by pipetting and kept at cold while in use. The NADH standard solution was stable for one week at 4°C and 1 month at -20°C. LDH Positive Control were reconstituted in 200 L of LDH Assay Buffer. We used 5 L of the prepared LDH Control as positive control. All samples and standards were run in duplicate. NADH Standards were added with volume of 0, 2, 4, 6, 8, and 10 L in duplicate into a 96 well plate, generating 0 (blank), 2.5, 5, 7.5, 10, and 12.5 nmole/well standards. After that, LDH Assay Buffer were added to a final volume of 50 L. Treated, control and Positive control cells (1106) were rapidly homogenized on ice in 500 L of cold LDH Assay buffer. Centrifugation at 10,000g for 15 min at 4°C was done in order to remove insoluble material. The soluble fraction of homogenate was used for assay. 20 L of samples were added into duplicate in 96 well plates. Then, the final volume of 50 L were done by adding LDH Assay Buffer. After that, the reaction mixture was mixed well using a horizontal shaker or by gentle pipetting. The absorbance at 450 nm at the initial time (A450) initial were measured. The plate was further incubated the plate at 37°C and measurements (A450) were taken every 5 min until the value of the most active sample is greater than the value of the highest standard (12.5 nmole/well). At this time the most active sample is near or exceeds the end of the linear range of the standard curve. The final measurement [(A450) final] for calculating the enzyme activity would be penultimate reading or the value before the most active sample is near or exceeds the end of the linear range of the standard curve. The time of the penultimate reading is T final.

Confocal Microscopy

The HaCat cells were maintained in KGM medium at 37°C, 5% CO_2 until 70% confluency and were seeded in chamber slides (Nunc,USA) at a density of 2×105 cells/well. The cells were treated with positive control (PMA), compound A, B, C, D and E for 24 hr. After incubation of 24 hr with treatment, the cells were fixed with 4% PFA for 15 min at room temperature, permeabilized in 0.1% Triton X100 for 5 min and blocked for 30 min in 2.5% BSA. The proteins of interest were then detected using, the caspase-3 rabbit polyclonal antibody (1:200 dilution) (apoptosis marker), followed by Alexa Fluor® 488-labelled goat anti-rabbit IgG (H+L) (1:1000 dilution, Invitrogen). The nucleus of HaCat cells were stained with 1mL of DAPI (10 mg/mL) and were visualized in cells treated under blue laser. Cell images were obtained using a confocal microscope (Olympus Cell Observer with an Infinity multibeam confocal scanning head) and mean florescence intensity were analyzed and quantified using Image.

Statistical Analysis

Each measurement was repeated in triplicates. Results are expressed as the mean + SD. We used one-way ANOVA, to compare the effects of various treatments with the untreated control cells. Different *p*-value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Results of Physicochemical Evaluation

The results of physicochemical parameters of all the plants were evaluated to ensure the quality of raw herb by comparing it with monograph mentioned in API. All the parameters are within the standard mentioned in API (Tables 1-5).

Results of Qualitative Phytochemical Estimation of Extracts

The obtained extract was further subjected to phytochemical screening for the detection of secondary metabolites present in them. The result of phytochemical screening are mentioned below in Table 6.

Results of *in vitro* assay

Results of MTT Assay

The HaCat cells were treated for 24 hr with compound A,B,C,D and E at concentration of 10 ug/mL. After the 24 hr of treatment

with these compounds, the viability of cells was significantly affected as shown in Figure 1. There was as significant difference between normal control as compared with positive control. In cells, treated with compound A were shown to have significantly decreased in mean viability of 45±3.9% (p value=0.0001). Similarly, the cells that were treated with compound B also were shown to have significantly decreased in mean viability of 55±3.4% (p value=0.0001) as compared to control cells. Also, the cells, which were treated with compound C, were also found to have significantly decreased in mean cell viability by 80.2±10.1%. However, cells treated with compound D and E have also decreased in cells viability by 86% but they were not found to be significant as compared to control cells. All the respective data are shown in Figure 4.

Results of LDH Assay

In this experiment, we assessed the LDH activity of cells treated with PMA, compound A, B, C, D and E after 24 hr. We have used (PMA) which was also known as 12-O-tetradecanoylphorbol 13-acetate.This is a specific activator of Protein Kinase C and hence activates nuclear factor-kappa B. NF-κB is a transcription factor that regulates numerous physiological functions and is involved in the pathogenesis of inflammations. PMA is the most common and potent phorbol ester. It is active at nanomolar concentrations and activates NF-κB in a dose-dependent manner. PMA is also possess high LDA and hence used as positive control. For calculating the LDH activity, we have measured the the change in measurement from $T_{initial}$ to T_{final} for the samples. A450=(A450)final-(A450)initial. The LDH activity of a sample may be determined by the following equation:

Table 1: **Physicochemical Evaluation of *C. longa*.**

Sl. No.	Parameters	Observation (%w/w) Avg Mean of triplicate analysis	API Limits
1.	Foreign matter.	Nil	NMT 2%
2.	Loss on drying.	--	-
3.	Total ash.	6.81	NMT 9 %
4.	Acid insoluble ash.	0.55	NMT 1%
5.	Water soluble extractive value.	13.04	NLT 12 %
6.	Alcohol soluble extractive value.	10.09	NLT 8%
7.	Heavy Metal Analysis		
Lead (Pb)		Not detected	NMT 10.0 ppm
Cadmium (Cd)		Not detected	NMT 3.0 ppm
Mercury (Hg)		Not detected	NMT 1.0 ppm
Arsenic (As)		Not detected	NMT 3.0 ppm

Table 2: **Physicochemical Evaluation of *P. pinnata*.**

Sl. No.	Parameters	Observation (%w/w) Avg Mean of triplicate analysis	API Limits
1.	Foreign matter.	Nil	NMT 3%
2.	Loss on drying.	---	
3.	Total ash.	2.41	NMT 3%
4.	Acid insoluble ash.	0.07	NMT 0.1%
5.	Water soluble extractive value.	20.18	NLT 13%
6.	Alcohol soluble extractive value.	25.41	NLT 23 %
7.	Heavy Metal Analysis		
Lead (Pb)		Not detected	NMT 10.0 ppm
Cadmium (Cd)		Not detected	NMT 3.0 ppm
Mercury (Hg)		Not detected	NMT 1.0 ppm
Arsenic (As)		Not detected	NMT 3.0 ppm

Table 3: **Physicochemical Evaluation of *P. corylifolia*.**

Sl. No.	Parameters	Observation (%w/w) Avg Mean of triplicate analysis	API Limits
1.	Foreign matter.	Nil	NMT 2 %
2.	Loss on drying.	---	---
3.	Total ash.	5.88	NMT 8 %
4.	Acid insoluble ash.	0.576	NLT 2 %
5.	Water soluble extractive value.	15.71	NLT 11 %
6.	Alcohol soluble extractive value.	19.09	NLT 13 %
7.	Heavy Metal Analysis		
Lead (Pb)		Not detected	NMT 10.0 ppm
Cadmium (Cd)		Not detected	NMT 3.0 ppm
Mercury (Hg)		Not detected	NMT 1.0 ppm
Arsenic (As)		Not detected	NMT 3.0 ppm

Table 4: **Physicochemical Evaluation of *W. fruticosa*.**

Sl. No.	Parameters	Observation (%w/w) Avg Mean of triplicate analysis	API Limits
1.	Foreign matter.	Nil	NMT 2 %
2.	Loss on drying.	---	---
3.	Total ash.	8.64	NMT 10 %
4.	Acid insoluble ash.	0.80	NMT 1 %
5.	Water soluble extractive value.	35.41	NLT 28 %
6.	Alcohol soluble extractive value.	17.28	NLT 7 %
7.	Heavy Metal Analysis		
Lead (Pb)		Not detected	NMT 10.0 ppm
Cadmium (Cd)		Not detected	NMT 3.0 ppm
Mercury (Hg)		Not detected	NMT 1.0 ppm
Arsenic (As)		Not detected	NMT 3.0 ppm

LDH Activity=BSample Dilution Factor (Reaction Time)V
B=Amount (nmole) of NADH generated between $T_{initial}$ and T_{final}.

Reaction Time=Tfinal-Tinitial (min).

V=sample volume (mL) added to well LDH activity is reported as nmole/min/mL=milliunit/mL. So, one unit of LDH activity is defined as the amount of enzyme that catalyzes the conversion of lactate into pyruvate to generate 1.0 mole of NADH per minute at 37°C. Hence, positive control (PMA) were shown to have highest LDH activity with mean±SD of 0.38±0.12 as shown in Figure 2. However, we observed significant reduction of LDH acitivity in cells treated with compound A by mean of 0.08±0.005 (p value=0.0001) as compared to positive control. Similarly, significant reduction of LDH activity in cells treated with compound B by mean of 0.11±0.05 (p value=0.0001) as compared to positive control. Also, the cells, which were treated with compound C and D were also found to have significantly decreased in mean LDH activity by (0.25±0.07) and (0.2±0.03) (p value=0.05) as compared to positive control. However, cells treated with compound E have also decreased in cellular LDH activity but they were not found to be significant as compared to control cells. All the respective data is shown in Figure 2. This shows that compound A and B have anti-inflammatory and reduced LDH activity.

Results of ELISA

In this experiment, we have also measure the protein expression of IL-12, IL-13 and IL-10 in cells treated with PMA and different compounds (A to E).

Table 5: **Physicochemical Evaluation of *A. indica*.**

Sl. No.	Parameters	Observation (%w/w) Avg Mean of triplicate analysis	API Limits
1.	Foreign matter	Nil	NMT 2%
2.	Loss on drying	---	---
3.	Total ash	6.33	NMT 7%
4.	Acid insoluble ash	0.46	NMT 1.5%
5.	Water soluble extractive value	16.58	NLT 5%
6.	Alcohol soluble extractive value	11.31	NLT 6%
7.	Heavy Metal Analysis		
	Lead (Pb)	Not detected	NMT 10.0 ppm
	Cadmium (Cd)	Not detected	NMT 3.0 ppm
	Mercury (Hg)	Not detected	NMT 1.0 ppm
	Arsenic (As)	Not detected	NMT 3.0 ppm

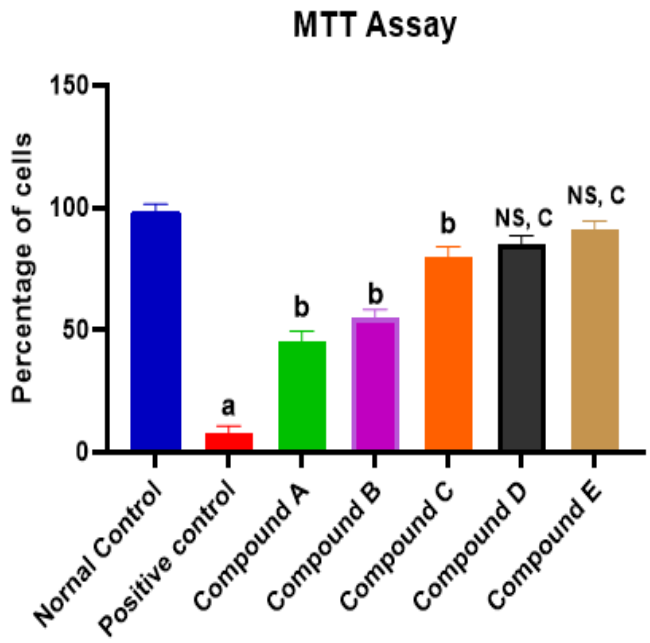

Figure 1: MTT assay was done to assess cell viability. Cells were treated with 10 µg/mL of compound A, B, C, D and E for 24 hr. Values for the data shown are representative of 3 experiments and are given as mean±SD. Statistical analysis was performed by oneway analysis of variance with all pairwise multiple comparison procedures done by Tukey test. Different *p*-value of less than 0.05 was considered statistically significant.

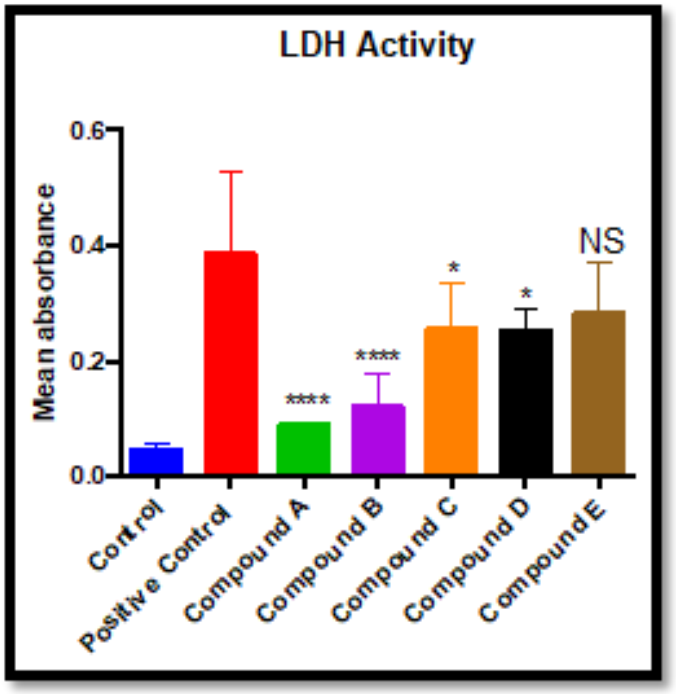

Figure 2: LDH assay was done to assess cellular inflammatory response. Cells were treated with 10µg/mL of compound A, B, C, D and E for 24 hr. Phorbol 12-myristate 13-acetate (PMA) used as positive control at 10 nM concentration. Values for the data shown are representative of 3 experiments and are given as mean±SD. Statistical analysis was performed by one-way analysis of variance with all pairwise multiple comparison procedures done by Tukey test. Multiple comparisons were done against positive control (PMA). Different *p*-value of less than 0.05 was considered statistically significant.

IL-12

IL-12 is pro-inflammatory cytokines that appears to regulate the inflammation. Here, in our study, we found significant reduction of IL-12 levels with mean value of 0.09±0.014 in cells treated with compound A as compared to cells treated with PMA (*p* value=0.0001). Similarly, significant reduction of IL-12 levels in cells treated with compound B by mean of 0.12±0.05 (*p* value=0.0001) as compared to positive control. Also, the cells, which were treated with compound C and D were also found to have significantly decreased in IL-12 levels mean by (0.35±0.11) and (0.28±0.05) (*p* value=0.0001) as compared to positive control. The cells treated with compound E have also significantly

Table 6: Results of qualitative phytochemical analysis of various extracts.

Chemical Constituents	Type of Extract	*C. longa*	*P. pinnata*	*P. corylifolia*	*W. fruticosa*	*A. indica*
Alkaloids	Acetone	+	+	+	+	+
	Ethanol	+	+	+	+	+
	Water	+	+	+	+	+
Tannins	Acetone	_	+	_	+	+
	Ethanol	+	+	+	+	+
	Water	+	+	+	+	+
Triterpenoids	Acetone	_	_	_	_	_
	Ethanol	+	_	+	+	+
	Water	+	+	+	+	_
Glycosides	Acetone	+	+	+	_	+
	Ethanol	+	+	+	+	+
	Water	+	+	+	+	+
Flavanoids	Acetone	+	+	+	+	_
	Ethanol	+	+	+	+	+
	Water	+	+	+	+	+
Carbohydrates	Acetone	+	+	+	+	_
	Ethanol	+	+	+	+	+
	Water	+	+	+	+	+
Proteins	Acetone	_	_	_		_
	Ethanol	+	+	+	+	+
	Water	+	+	_	+	+

decreased in IL-12 levels mean by (0.28±0.07) (p value=0.0001) as compared to positive control All the respective data is shown in Figure 3(A). This shows that compound A and B have reduced IL-12 levels and hence are less pro-inflammatory.

IL-13

IL-13 cytokines is majorly responsible for inhibiting the pro-inflammatory cytokine and chemokine production *in vitro* and has known to possess the potent anti-inflammatory activities. IL-13 is an anti-inflammatory cytokine that plays a unique role in the induction and maintenance of IgE production and IgE-mediated allergic responses. Here, in our study, we found significant increase in IL-13 levels with mean value of 0.5±0.06 in cells treated with compound A as compared to cells treated with PMA (p value=0.0001). Similarly, significant up regulation of IL-13 levels in cells treated with compound B by mean of 0.42±0.12 (p value=0.0001) as compared to positive control. Also, the cells, which were treated with compound C and D were also found to have significantly slight increase in IL-13 levels mean by (0.28±0.07) and (0.21±0.06) (p value=0.01) as compared to positive control. The cells treated with compound E have also slight increase in IL-10 levels mean by (0.24±0.07) (p value=0.05) as compared to positive control but they are not significant. All the respective data is shown in Figure 3(B). This shows that compound A and B have high IL-13 levels and hence exhibit anti-inflammatory response.

IL-10

IL-10 cytokine were considered to be potent anti-inflammatory cytokine that plays a central role in limiting host immune response to pathogens, thereby preventing damage to the host and maintaining normal tissue homeostasis. Here, in our study, we found significant increase in IL-10 levels with mean value of 0.5±0.05 in cells treated with compound A as compared to cells treated with PMA (p value=0.0001). Similarly, significant up regulation of IL-10 levels in cells treated with compound B by mean of 0.47±0.08 (p value=0.0001) as compared to positive control. Also, the cells, which were treated with compound C and D were also found to have slight increase in IL-10 levels mean by (0.28±0.06) (p value=0.01) and (0.18±0.09) as compared to positive control. The cells treated with compound D have also significantly slight increase in IL-10 levels mean by (0.16±0.06) (p value=0.05) as compared to positive control All the respective data is shown in Figure 3(c). This shows that compound A and B have high IL-10 levels and hence exhibit anti-inflammatory response.

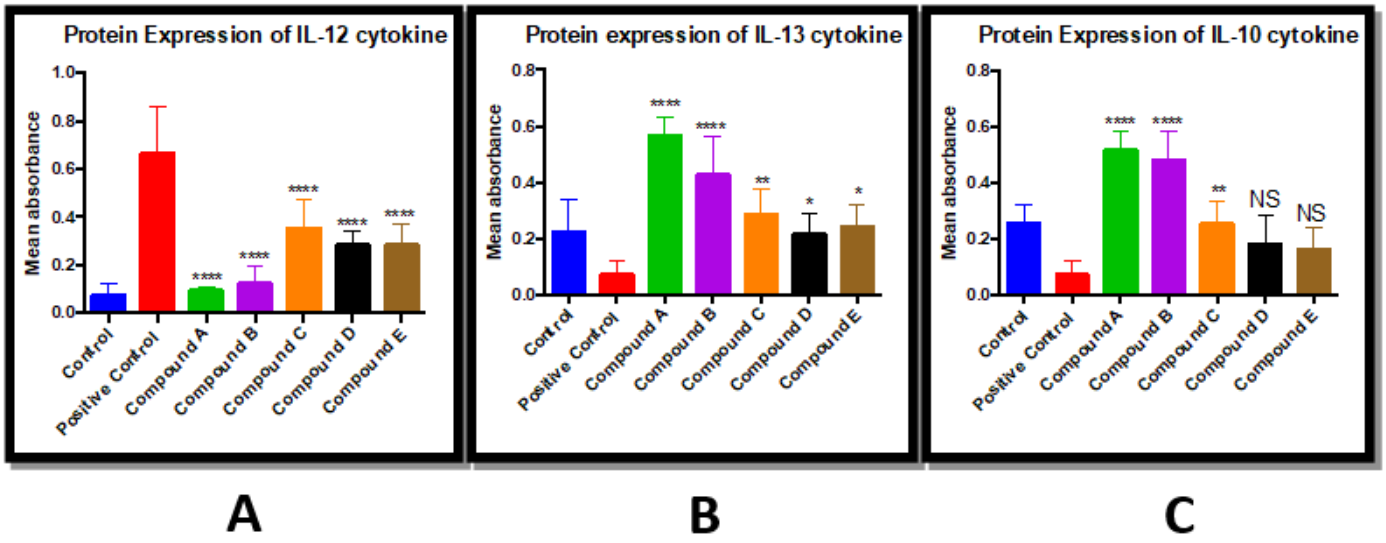

Figure 3(A-C): ELISA of different cytokines were done to quantitate the protein expression of IL-12, IL-13 and IL-10. Cells were treated with 10µg/mL of compound A, B, C, D and E for 24 hr. Phorbol 12-myristate 13-acetate (PMA) used as positive control at 10 nM concentration. Values for the data shown are representative of 3 experiments and are given as mean±SD. Statistical analysis was performed by one-way analysis of variance with all pairwise multiple comparison procedures done by Tukey test. Multiple comparisons were done against positive control (PMA). Different p-value of less than 0.05 was considered statistically significant.

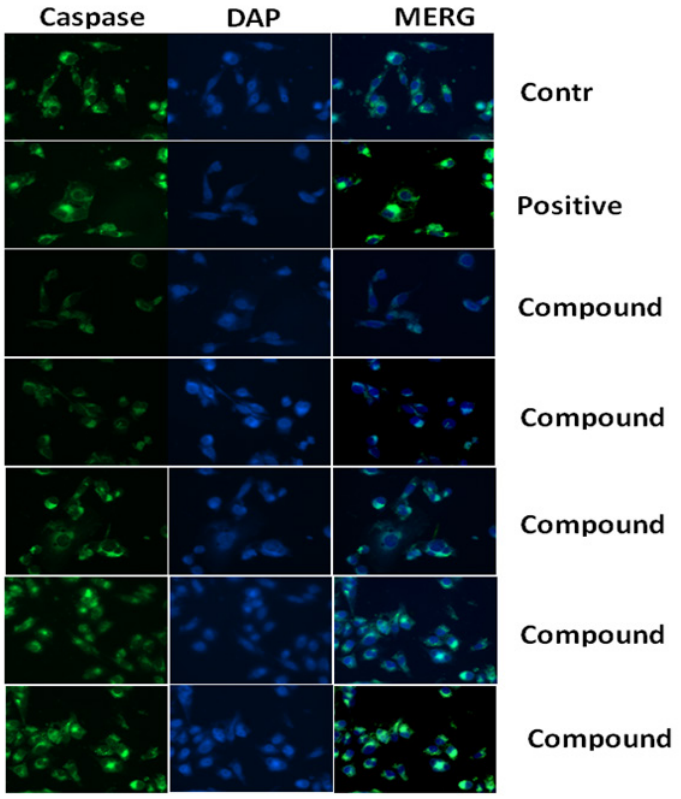

Figure 4: We have also calculated the mean fluorescence intensity of confocal images of cells treated with PMA, compound A, B, C, D and E. The expression of Caspase 7 were shown to be significantly down regulated in cells treated with compound A and B with mean value of (39.5±5.2)% and (52.3±6.0)% as compared to control cells. Also, the cells, which were treated with compound C, were also found to have decreased in Caspase 7 expression by 74±6.6%. However, cells treated with compound D and E have also decreased in Caspase 7 expression by 76% but they were not found to be significant as compared to control cells.

Results of Confocal Microscopy of Caspase 7

We have also examined the effect of 10µg/mL of compound A, B, C, D and E for 24 hr on Caspase 7localization and expression through immunefluorescence. The immunofluorescence of treated cells was done, target protein localization and expression was analyzed using confocal microscopy. We have observed weak cytoplasmic expression of Caspase 7protein in case of compound A and B, but intense cytoplasmic expression in positive control as compared to control cells (Figure 4). Similarly, the cytoplasmic expression of Caspase 7 was decreased in cells treated with C, D and E but they seems similar to control (Figure 4).

Further, we have also calculated the mean fluorescence intensity of confocal images of cells treated with PMA, compound A, B, C, D and E. The expression of Caspase 7 were shown to be significantly down regulated in cells treated with compound A and B with mean value of (39.5±5.2)% and (52.3±6.0)% as compared to control cells. Also, the cells, which were treated with compound C, were also found to have decreased in Caspase 7 expression by 74±6.6%. However, cells treated with compound D and E have also decreased in Caspase 7 expression by 76% but they were not found to be significant as compared to control cells. All the respective data are shown in Figure 4.

CONCLUSION

The findings presented in this study hold significant implications in the context of understanding and potentially utilizing the antipsoriatic properties of traditional Indian medicinal plants. The discussion of these results can be structured as follows:

Traditional Medicinal Use

The traditional use of *Curcuma longa, Azadirachta indica, Psoralea corylifolia, Pongamia pinnata*, and *Woodfordia fruticosa* in the treatment of various ailments, including psoriasis, has been supported by the findings of this study. The promising antiproliferative activity of these plants on skin keratinocytes suggests that the traditional knowledge and practices of using these botanicals for healing purposes are well-founded. Incorporation of this herbal medicine may plays a major role for the treatment of various dreadful disease such as psoriasis.

Phytochemical and Physicochemical Analysis

While the primary focus of this study was to evaluate the antipsoriatic potential of these plant extracts, it is worth noting that a preliminary physicochemical and phytochemical analysis was also conducted. The identification of key bioactive compounds within these plants could be essential for future research and the development of more targeted treatments for psoriasis.

Variation in Extract Efficacy

The study revealed varying levels of anti-proliferative activity among the different plant extracts. Compounds A, B, and C displayed a significant decrease in cell viability compared to the control group, indicating their potential as more potent anti-psoriatic agents. In contrast, compounds D and E did not exhibit significant differences compared to the control. This variation in efficacy underscores the importance of further investigation into the specific compounds responsible for the observed effects.

Clinical Potential

Psoriasis is a chronic skin condition with limited treatment options. The positive outcomes of this study suggest that these Indian medicinal plants could serve as valuable resources in the development of effective antipsoriatic treatments. Clinical trials and further research are warranted to better understand their potential benefits and safety in real-world applications. Incorporation of these extracts in Novel drug delivery system may lead to great therapeutic potential.

Future Research Directions

The findings of this study open the door to various promising avenues for future research. These include isolating and identifying the active compounds responsible for the observed anti-proliferative effects, conducting animal studies to validate the *in vitro* findings, and eventually progressing to human clinical trials. Additionally, research on the safety and long term effects of these plant extracts should be a priority.

In conclusion, this study not only reaffirms the traditional use of *Curcuma longa, Azadirachta indica, Psoralea corylifolia, Pongamia pinnata*, and *Woodfordia fruticosa* for the treatment of psoriasis but also provides a solid foundation for further investigations that may lead to the development of novel and more effective treatments for this chronic skin condition. The varying levels of efficacy among the different plant extracts highlight the need for continued research into their specific mechanisms of action and potential synergies in treating psoriasis.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

LDH: Lactate dehydrogenase; **MTT:** (3-[4,5-dime thylthiazol-2-yl-2,5 diphenyl tetrazolium bromide]); **HaCaT:** Human Epidermal Keratinocyte; **DMSO:** Dimethyl sulfoxide; KGM: Keratinocyte culture growth medium; **NCC:** National centre for cell science; **PMA:** Phorbol 12- myristate 13 acetate; **hr:** Hour; **ANOVA:** Analysis of variance; **API:** Ayurvedic Pharmacopoeia of India; **PKC:** Pritein kinase; **IL:** Interleukin; **ELISA:** Enzyme linked immune-sorbate assay; **DAPI:** Diamidino-2- phenyliudole; **LDA:** Lactate dehydrogenase activity.

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