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Research Article

INVITRO ANTIPSORIATIC AND INHIBITORY EFFECTS OF ETHYL ACETATE AND METHANOL FRACTION OF *CASSIA SOPHERA* AND *MALLOTUS PHILIPPINENSIS* ON INFLAMMATORY CYTOKINES

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ABSTRACT

The plants *Cassia sophera* and *Mallotus philippinensis*, traditionally, is claimed to be useful in the treatment of psoriasis. In order to evaluate antipsoriatic activity of ethyl acetate and methanol fraction of *Cassia sophera* and *Mallotus philippinensis* was investigated using HaCaT keratinocytes cell inhibition and cytokine inhibition assay. Further, the total alkaloids, steroids and flavonoids present in the ethyl acetate and methanol fraction of *Cassia sophera* and *Mallotus philippinensis* were quantified using standard procedures. The cytotoxic effect of ethyl acetate and methanol fraction of *Cassia sophera* and *Mallotus philippinensis* were evaluated using HaCaT cells, a rapidly multiplying human keratinocyte cell line, as a model of epidermal hyperproliferation in psoriasis. The tested fractions showed appreciable antiproliferant activity in HaCaT cell line. Tested fractions showed mild to moderate significant inhibitory effects on IL-lα, IL-1β, IL-6, IL-8, IL-17 and TNF-αin cytokine inhibition assay. These are key cytokines involved in the pathogenesis of psoriasis at higher concentration. We concluded, using *invitro* antipsoriatic model, that the ethyl acetate and methanol fraction of *Cassia sophera* and *Mallotus philippinensis* have significant antipsoriatic activity. Therefore it can be effective and safety as dietary supplements with health benefits to psoriatic patients.

Key words: *Invitro*antipsoriatic, HaCaT keratinocytes cell, Cytokine Inhibition Assay.

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INTRODUCTION

Psoriasis is a chronic inflammatory skin disorder characterized by rapid proliferation of keratinocytes and incomplete keratinization. Affordability, availability and side effects of prolonged use of allopathic drugs still remain a challenge and concern in the treatment of psoriasis. Discovery of safer and more effective anti-psoriatic drugs remains an area of active research at the present time. As psoriasis is an immune disorder and also associated with over expression of proinflammatory cytokines and abnormal proliferation of keratinocytes, therapeutic agents that either modulate the immune system or normalize the differentiation program of psoriatic keratinocytes are suggested for treating psoriasis which includes topical steroids, retinoids and immunomodulatory agents. Medicinal plants and their active components have been shown to be an important source of immunomodulators. Steroids are usually the very first treatment used for different types of psoriasis. Steroids are very effective at reducing inflammation and when used properly result in few side effects, making them the obvious choice for a first try by dermatologists on patients newly-diagnosed with psoriasis. A large number of flavonoids have been shown to be potential immunomodulators, acting as anti - inflammatory, anti stress, anticancer agents and in various skin diseases.

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Traditional medicines hold a great promise as source of easily available effective therapy for skin diseases to the people, particularly in tropical developing countries including India. Herbal remedies for psoriasis are increasingly popular and main stream. Moreover usage of chemicals in cosmetics, unpurified drinking water pesticides in foods, carcinogenic chemicals in shampoos are key factors for psoriasis which is highly unavoidable in future. This type of scientific findings will quench the thirst of newer traditional drugs. The plants *Cassia sophera* species have been well known for their laxative and purgative properties and for the treatment of skin diseases and leaves of *Mallotus philippinensis* species are known to contain different natural compounds, mainly phenols, diterpenoids, steroids, flavonoids, cardenolides, triterpenoids, coumarins, isocoumarins, and many more especially phenols; that is, bergenin, mallotophilippinens, rottlerin, and isorottlerin have been isolated, identified, and reported interesting biological activities such as antimicrobial, antioxidant, antiviral, cytotoxicity, antioxidant, skin disorders, anti-inflammatory, immunoregulatory activity protein inhibition against cancer cell (Aldeen T, Basra M, 2011; Lowes *et al*., 2007). Keeping the above facts as an idea, the present study will be aimed to establish scientific data for the anti-psoriatic activity of steroid fraction and flavonoid fraction from the *leaves of Cassia sophera* and *Mallotus philippinensis* in various proportions and to establish a new polyherbals for the management of psoriasis.

MATERIALS AND METHODS

Selection of Indian medicinal plants

The Indian medicinal plants [leaves of *Cassia sophera* (Family: cesalpinaceae), *leaves of Mallotus philippinensis* (Famil: 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.

Collection and Authentication of selected plants:

The Indian medicinal plants [*Cassia sophera*(Family: cesalpinaceae), *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.

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 leaf 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 desiccator and it is used for preliminary phytochemical analysis.

Preliminary Phytochemical analysis of different extracts of *Ipomoea staphylina*

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.

Test for alkaloids:

- Small quantities of hexane, chloroform, ethyl acetate and methanolic fractions of plant extracts were separately treated with few drops of dilute hydrochloric acid and filtered. The filtrates were then treated with Dragendroff's reagent (Sodium iodide, basic bismuth carbonate, glacial acetic acid and ethyl acetate). Development of orange brown precipitate in chloroform and methanol fractions indicated the presence of alkaloids.
- Small quantities of hexane, chloroform, ethyl acetate and methanolic fractions of plant extracts were separately treated with few drops of dilute hydrochloric acid and filtered. The filtrates were treated with Wagner's reagent (Iodine and potassium iodide). Development of reddish brown precipitate in chloroform and methanol extracts suggested the presence of alkaloids.
- Small quantities of hexane, chloroform, ethyl acetate and methanolic extracts of plant extracts were separately treated with few drops of dilute hydrochloric acid and filtered. The filtrates were treated with Hager's reagent (Aqueous solution of picric acid). Formation of yellowish precipitate in chloroform and methanol fractions demonstrated the positive response for alkaloids.

Test for reducing sugar:

- Small quantities of hexane, chloroform, ethyl acetate and methanolic fractions of Plant extract were separately dissolved in minimum amount of distilled water and filtered. To the filtrate equal volume of Benedict's reagent were mixed in a test tube separately and heated for few minutes. Formation of brick red precipitate in methanol and aqueous extracts confirmed the presence of reducing sugars.
- Small quantities of hexane, chloroform, ethyl acetate

and methanolic fractions of Plant extracts were separately dissolved in minimum amount of distilled water and filtered. To the filtrate equal volume Fehling's solutions in a test tube separately heated for few minutes. Development of brick red color in methanolic fraction demonstrated the presence of reducing sugars.

Test for glycosides:

A pinch of extract was taken in a watch glass and 2 drops of alcohol was added to dissolve the extract. An equal quantity of anthrone was added and mixed thoroughly and dried. Then one drop of concentrated sulphuric acid was added, spreaded in a thin film with a glass rod in a watch glass and heated over the water bath. Formation of dark green colour confirms the presence of glycosides

Anthraquinone glycosides (Modified Borntrager's test):

- About 10 mg ofhexane, chloroform, ethyl acetate and methanolic fractions of plant extracts were boiled with dilute hydrochloric acid for 5 minutes and few drops of ferric chloride solution were added, filtered while hot and cooled. The filtrate was then extracted with benzene and the benzene layer was separated. Equal volume of dilute ammonia solution was added to the benzene extract and shaken well. Rosy pink colour was not observed in the ammonia layer showing the absence of anthraquinone glycosides.

Cardiac glycosides (Keller-Kiliani test):

- About 10 mg of hexane, chloroform, ethyl acetate and methanolic fractions of plant extracts were boiled with 70% alcohol for 3 minutes and filtered. To the filtrate 5 ml of water and 0.5 ml of a strong solution of lead acetate were added, shaken well and filtered. The clear filtrate was treated with equal volume of chloroform and chloroform layer was evaporated. The residue was dissolve in 3 ml of glacial acetic acid and to this two drops of ferric chloride solution were added. The contents were transferred to a test tube containing 2 ml of concentrated sulphuric acid. Brown colour ring was observed indicating the presence of cardiac glycosides.

Cyanogenetic glycosides (Griard's test):

- Small quantity of hexane, chloroform, ethyl acetate and methanolic fractions of plant extracts *were* placed in a stoppered conical flask with just sufficient water to cover it. A sodium picrate paper strip was inserted through the stopper so that it was suspended in the flask and it was set-aside for 2 hours in a warm place. No change in the color of the sodium picrate paper was observed indicating the absence of cyanogentic glycosides.

Test for phytosterols:

Libermann-Burchard reaction:

- 10 mg of hexane, chloroform, ethyl acetate and methanolic fractions of *Plant extract* were separately dissolved in 1 ml of chloroform; 1 ml of acetic anhydride was then added to the mixture followed by 2 ml of concentrated sulphuric acid. A reddish violet ring at the junction of the two layers in hexane and methanolic extracts confirmed the presence of steroids.

Salkowski test:

- When concentrated sulphuric acid and chloroform were added to hexane, chloroform, ethyl Acetate and methanol fractions of plant extracta reddish-blue colour was produced in the chloroform layer and green fluorescence in acid layer, of hexane and methanolic extracts suggesting the presence of steroids.

Tests for saponins:

- Small quantities of hexane, chloroform, ethyl acetate and methanolic fractions of plant extracts dissolved separately in minimum amount of distilled water and shaken in a graduated cylinder for 15 minutes. Absence of stable foam in all the fractions suggested the absence of saponins

Test for tannins:

- Small quantities of hexane, chloroform, ethyl acetate and methanolic fractions of plant extract dissolved in minimum amount of distilled water and filtered. The filtrate treated with 10% aqueous potassium dichromate solution. yellowish brown precipitate in all the fractions demonstrated the presence of tannins
- Small quantities of hexane, chloroform, ethyl acetate and methanolic fractions of Plant extract dissolved in minimum amount of distilled water and filtered. The filtrate was allowed to react with 10% aqueous lead acetate solution. Yellow colour precipitate formation in all the fractions indicated the presence test for tannins.
- Small quantities of hexane, chloroform, ethyl acetate and methanolic fractions of Plant extract dissolved in minimum amount of distilled water and filtered. The filtrates are then allowed to react with 1 ml of 5% ferric chloride solution. Greenish black colour in all the fractions indicates the presence of tannins.

Test for proteins and free amino acids:

- Small quantities of hexane, chloroform, ethyl acetate and methanolic fractions of Plant extract were dissolved in a few ml of distilled water separately and treated with Ninhydrin (Triketohydrindene hydrate) at the pH range of 4 to 8. The purple coloration suggested the presence of amino acids.
- Small quantities of hexane, chloroform, ethyl acetate and methanolic fractions of Plant extract were dissolved in a few ml of distilled water separately and treated with Millons reagent. The formation of purple coloration in

methanol and aqueous extracts suggested the presence of amino acids.

- Small quantities of hexane, chloroform, ethyl acetate methanolic fractions of Plant extract were treated withequal volume of 5% NaOH and 1%$CuSO_4$ solution, and colour change was observed in methanolic extract indicating the presence of proteins and free amino acids.

Test for gums and mucilage:

Small quantities of hexane, chloroform, ethyl acetate and methanolic fractions of Plant extract dissolved in minimum amount of distilled water and filtered. The filtrate was treated with equal volume of concentrated sulphuric acid. Then, it was treated with 15% alcoholic solution of α-naphthol (Molish's reagent). Formation of red-violet ring at the junction of sulphuric acid layer and in methanol fraction indicated the positive test for gums (Molish's test).

Test for flavanoids:

Shinoda Test

To the of hexane, chloroform, ethyl acetate and methanolic fractions of Plant extract, magnesium turnings and few drops of concentrated hydrochloric acid were added and boiled for 5 minutes. Red colour was produced in ethyl acetate, methanolic fractions showing the presence of flavanoids.

Alkali test:

- Small quantities of hexane, chloroform, ethyl acetate and methanolic fractions of Plant extract were dissolved in ethanol separately and was hydrolyzed with 10% sulphuric acid and cooled. Then, it was extracted with diethyl ether and divided into three portions in three separate test tubes for each extract. 1ml of diluted sodium carbonate, 1ml of 0.1M sodium hydroxide and 1ml of diluted ammonia solution were added to the first, second and third test tubes of both extracts respectively. Development of deep yellow colour produced in ethyl acetate, methanolic fractions showed the presence of flavanoids.

Tests for fixed oil and fats:

Spot test:

- Small quantities of hexane, chloroform, ethyl acetate and methanolic fractions of Plant extract various extracts were passed separately between the filter paper leaves stain in all the fractions. Indicates the presence of oils and fats

Saponification test:

- Few drops of 0.5N alcoholic potassium hydroxide were added to all fractions with few drops of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. Characteristic change was observed in all the fractions which indicated the presence of oils and fats.

ESTIMATION OF PHYTOCONSTITUENTS

Estimation of Steroids

1ml of test sample of steroid solution was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70±20° C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank.

Determination of Alkaloid

The plant extract (1mg) was dissolved in dimethyl sulphoxide (DMSO), added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/g of extract (Fazel Shamsa *et al*., 2008).

Determination of total Flavonoid content

The total flavonoids determined by complementary colorimetric methods, aluminum chloride method and the values obtained were summed up to give the final value. The method was performed for ethyl acetate and methanol fraction of *Cassia sophera*and *Mallotus philippinensis*I) Aluminum chloride colorimetric method Quercetin was used to make the calibration curve.

1 mg of Quercetin was dissolved in 100 ml ethanol to produce (10 µg/ml). From this solution 0.1, 0.2, 0.3, 0.5, 0.8, 1 ml taken and diluted up to 10 ml ethanol to produce 1, 2, 3, 5, 8, 10µg/ml concentrations respectively. The standard solution was separately mixed with 1 ml of ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of reaction mixture was measured at 415 nm with Shimadzu 1800 spectrophotometer. The amount of 10% aluminum chloride was substituted by the same amount of distilled water in blank. About 1.0 ml of extract solution containing 4 mg extracts was reacted with Aluminum chloride for determination of flavonoids content as described in above procedure; the percentage of total flavonoids was calculated from calibration curve (Maurya& Singh, 2010).

***IN VITRO* ANTI-PSORIATIC ACTIVITY**

***In-vitro* anti-psoriatic activity**

Keratinocyte is the primary cell found in the epidermis, the outermost layer of the skin constituting 90% of the cells. The function of the keratinocyte is the formation of the keratin layer that protects the skin and the underlying tissues from the environmental damages such as the heat, UV. Anti-psoriatic activities are reflected by inhibition of keratinocyte proliferation. Hence the potency of the ethyl acetate and methanolic extract of plant material were screened using HaCaT human keratinocyte cell line.

HaCaT keratinocytes cell inhibition assay

*In vitro*antipsoriatic activity will be carried out in HaCaT human keratinocyte cell line. Human HaCaT keratinocytes will be obtained from NCCS, Pune, India. The cells will be seeded at a concentration of 1.0×105 cells/ml in a 96 well microtitre plate and grown in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (BioWest). After 24 h, the supernatant will be decanted and the monolayer will be washed once. Then 100µl of test substance in various concentrations will be added to the cells in microtitre plates. Test compounds will be prepared in dimethyl sulphoxide (DMSO) and then diluted with DMEM; the final concentration of DMSO will be 0.2% in the culture medium. Each sample concentration will be tested in triplicates. Controls will be performed with DMSO or medium alone. Asiaticoside (Sigma) will be used as positive control. The plates will be then incubated at 37°C for 3 days in 5% CO_2 atmosphere. Anti-proliferantactiviy will be assessed by performing the Sulphorhodamine B (SRB) assay. Cells will be fixed by adding 25µl of ice-cold 50% trichloroacetic acid on top of the growth medium and the plates will be incubated at 4°C for 1 h, after which plates will be washed to remove traces of medium, drug and serum. SRB stain (50µl; 0.4% in 1% acetic acid) (Sigma) will be added to each well and left in contact with the cells for 30 min after which they will be will held with 1% acetic acid, rinsing 4 times until only dye adhering to the cells will be left. The plates will be then dried and 100µl of 10mM Tris buffer (Sigma) added to each well to solubilise the dye. The plates will be shaken gently for 5min and absorbance read at 550nm using a micro plate reader (Vjayalakshmi *et al*., 2012).

CYTOKINE INHIBITION ASSAY

Assay for the inhibitory effects on IL-l*α*, IL-1β, IL-6, IL-8, IL-17 and TNF-α biosynthesis

Endotoxin (LPS) from Escherichia coli 055:B5 was obtained from Difco (Detroit, MI). Heparin was purchased from Takeda (Osaka, Japan) and ELISA kits from RayBio® (RayBiotech, Inc.).

Blood collection

About 20 mL of blood collected from healthy man volunteers after an overnight fast of 10–12 h. containing 20U heparin/ml by venapuncture and 30% solution is prepared by suspending in supplemented RPMI-1640 medium containing 100 U/ml penicillin and 100 mg/ml streptomycin.

Procedure

Lipopolysaccharide stimulated human peripheral mononuclear cells (LPS) (1 µg/ml) was dissolved in the supplemented RPMI-1640 media at a concentration of 3 µg/ml. The test sample was dissolved in DMSO at concentrations of 1, 3, 10, and 30 µg/ml and each of these concentrations was diluted with the supplemented RPMI-1640 media (1:100). Only DMSO was contained in control suspension. Equal volumes from each of three solutions (whole blood, LPS and test sample) were mixed and the mixture was incubated at 37°C in a humidified atmosphere of 5% CO_2–95% air for 18—24 h. The supernatant of culture prepared by centrifugation was stored at -20°C until the assay of cytokine. The concentrations of the human cytokines (IL-lα, IL-1β, IL-6, IL-8, IL-17, TNF-α) were assayed using an ELISA kits.[18] The ratio (%) of inhibition of the cytokine release was calculated by the following equation:

Inhibition (%) = 100×(1-T/C)

Where*T* represents the concentration of the cytokine in the culture supernatant with the test compound, and *C* represents the concentration of the cytokine in the culture supernatant with the solvent (control).

RESULTS AND DISCUSSION

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 *Mallotus*

philippinensis 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 (Hanane El Hajaji *et al*., 2010).

HaCaT keratinocytes cell inhibition assay

The cytotoxic effect of ethyl acetate and methanol fraction of *Cassia sophera* and *Mallotus philippinensis* were evaluated using HaCaT cells, a rapidly multiplying human keratinocyte cell line, as a model of epidermal hyperproliferation 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) inHaCaT cell line. Methanol fraction of *Cassia sophera* and *Mallotus philippinensis* 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.

Inhibitory effects of the test samples on IL-lα, IL-1β, IL-6, IL-8, IL-17, TNF-α biosynthesis are given in Table 1 as the inhibitory percentages. For the interpretation of the results, percentage values are classified under four groups; an inhibition between 70 and 100% is accepted as high, values between 40 and 69% as moderate, 20 and 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 (Nickoloff *et al*., 1991).

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

Table 1. Assay for the inhibitory effects on IL-lα, IL-1β, IL-6, IL-8, IL-17 and TNF-α biosynthesis

Treatment	Final Concentration (µg/ml)	Inhibitory ratio (%)					
		IL-lα	IL-1β	IL-6	IL-8	IL-17	TNF-α
Ethyl acetate fraction of *Cassia sophera* and *Mallotus philippinensis*	1	0	1	4	2	4	8
	5	5	8	14	9	11	18
	10	9	15	26	16	23	38
	20	16	24	39	35	44	72
Methanolic fraction of *Cassia sophera* and *Mallotus philippinensis*	1	0	2	4	3	4	10
	5	7	9	16	10	13	19
	10	12	19	29	18	24	41
	20	18	28	42	38	46	76

CONCLUSION

The present investigation aims at the development of potent phytomedicine from ethyl acetate and methanol fraction of *Cassia sophera* and *Mallotus philippinensis* for the treatment of psoriasis. The result of the study authentifies the folk lore claim in the use of aerial part of *Cassia sophera* and *Mallotus philippinensis* in traditional medicine for the treatment of psoriasis. Further studies are in process for identification of possible mechanisms of its anti-psoriatic property.

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