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Research Article

**PHYTOCHEMICAL SCREENING AND PHARMACOLOGICAL
ACTIVITIES OF HYDROALCOHOLIC EXTRACT OF
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Abstract:

Before there were contemporary therapies, herbal medicines were employed to treat acne and other skin disorders. Even though there hasn't been much research on many herbal remedies, there is a tonne of anecdotal evidence. Because of their superior therapeutic effect and lower toxicity, herbal medicines are better. They lessen toxicity by reducing the frequency of doses. Natural medications from active plant extracts, essential oils, phytomolecules, and extracts are included in herbal medicines. In light of this, the current study's objective is to evaluate the anti-acne potential of the medicinal plant *Sterculia lychnophora*. The ascorbic acid and extracts have demonstrated dose-dependent DPPH radical scavenging. The IC50 values for the standard and extracts for radical scavenging were 19.29 and 88.37, respectively, for ascorbic acid > extract. *Propionibacterium acnes*. The *Sterculia lychnophora* hydroalcoholic extract's also showed the potent effect against diabetes. According to these documented data, hydroalcoholic extract exhibits strong anti-acne efficacy. The current study came to the conclusion that *Sterculia lychnophora* has important secondary metabolites that are in charge of its varied pharmacological functions. It also demonstrated that the *Sterculia lychnophora*'s metabolites may be extracted most successfully using the polar solvent hydroalcoholic.

Key words: *Sterculia lychnophora*, *Propionibacterium acnes*, Antioxidant, antidiabetic, Anti acne**Corresponding author:****Priya Yadav,**

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INTRODUCTION:

Acne is a chronic inflammatory skin disease involving the sebaceous glands. Four major pathogenesis are involved in the development of androgen-induced increased sebum hyperproduction, altered follicular keratinization, inflammation and *Propionibacterium acnes* (Williams & Dellavalle, 2012). It is also affected by environmental pollution, social environment, changes of dietary structure and lifestyle, for example, worsening air pollution, the intake of sweets, staying up late, social network and social media. Thus, the prevalence of acne increases year by year. According to a systematic analysis for the Global Burden of Disease Study, in 2010, the prevalence of acne among all the population in the world was 9.38%, ranking the eighth in the world. From 2006 to 2016, the prevalence of acne increased by 5.1% (Dreno, 2017).

Acne most often affects the face, but it may spread to involve the neck, chest and back, and sometimes even more extensively over the body.

Individual lesions are centred on the pilosebaceous unit, ie the hair follicle and its associated oil gland. Several types of acne spots occur, often at the same time. They may be inflammatory papules, pustules and nodules; or non-inflamed comedones and pseudocysts (Radtke *et al.*, 2010)

Topical or/and systematic treatments are used to treat acne. The response of patients to treatment is considerably different. Usually more than one treatment modality is employed to treat acne and best results are achieved when treatments are individualized on the basis of clinical evaluations. Normally used topical and oral antibiotics are Clindamycin, Erythromycin, Triclosan Tetracycline, Minocycline and Metronidazole. Retinoids are the mainstay of therapy in patients who only have comedones. They are capable of reducing inflammatory lesions and the number of comedones (40% - 70%). Other agents, including isotretinoin, oral antibiotics, topical antimicrobials, and hormonal therapy, have been shown to yield high response rates. Patients with mild to moderate severity, inflammatory acne with papules and pustules are recommended to be treated with topical antibiotics combined with retinoids.

Natural products derived from plant, animal and mineral sources have the capacity to treat different human diseases. Around 80% of the general population uses natural products for the treatment of different diseases whereas allopathic drugs may cause many side effects. Acne causing bacteria

becomes resistant to the drugs if they are used for a long period. Due to low toxicity and side effects, herbal medicine is becoming popular when compared to allopathic (Dey *et al.*, 2014).

Herbal remedies were used to clear up acne and other skin conditions well before modern treatments existed. Despite the lack of research on many herbal solutions, anecdotal evidence is plentiful. Herbal remedies tend to have fewer side effects than modern treatments. Some herbs have antibacterial, anti-inflammatory, and antiseptic properties. These properties may help reduce acne-causing bacteria and inflammation, and heal blemishes. Herbal medicines better because of their less toxicity and better therapeutic action. They reduce toxicity by decrease dose frequency. Herbal therapies include naturally derived drugs from active plant extracts, essential oils, phytochemicals and extracts. Keeping this view the aim of the present study is to test anti acne potential of medicinal plant *Sterculia lychnophora*.

MATERIAL AND METHODS:**Material:**

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals and solvent used in this study were of analytical grade. The pathogenic microbes used in the current study are obtained from Microbial Culture collection, National Centre For Cell Science, Pune, Maharashtra, India.

Methods:**Collection of plant material:**

The plants have been selected on the basis of its availability and folk use of the plant. The fruits of *Sterculia lychnophora* were collected from local area of Bhopal in the month of February, 2022. Drying of fresh plant parts was carried out in sun but under the shade. Dried fruits of *Sterculia lychnophora* were preserved in plastic bags, closed tightly and powdered as per the requirements.

Defatting of plant material:

56.83 gram shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process:

Defatted powdered of *Sterculia lychnophora* has been extracted with ethanol solvent using maceration

process for 48 hrs, filtered and dried using vacuum evaporator at 40°C (Mukherjee, 2007).

Determination of percentage yield:

The extraction yield is an assessment of the efficiency of the solvent in extracting bioactive components from the selected natural plant samples and was defined as the quantity of plant extracts

recovered after solvent extraction compared to the original quantity of plant samples. The yield of the collected plant extracts was measured in grams after extraction, and then converted into percentage. For calculating the percentage yield of selected plant products, formula following was introduced. By using the following formula the percentage yield of extract was calculated:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powdered drug}} \times 100$$

Phytochemical Screening:

Medicinal plants are traditional pharmaceutical commodities and many of the current medicinal drugs are derived indirectly from plants. Phytochemical materials consist of two main bioactive components (chlorophyll, vitamins, amino acids, sugar etc.) and secondary bioactive components; (alkaloids, terpenoids, phenols, flavonoids etc.). Phytochemical analyses were performed according to the normal protocols for extract. Phytochemical examinations were carried out for all the extracts as per the standard methods (Kokate, 1994).

Quantitative estimation of bioactive compounds:

Total phenolic content estimation:

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method (Gaur Mishra *et al.*, 2017). 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50 µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Total flavonoids content estimation:

Determination of total flavonoids content was based on aluminium chloride method (Parkhe and Bharti,

2019). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 10-50 µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2% AlCl₃ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

Antioxidant activity of *Sterculia lychnophora* using DPPH method:

DPPH scavenging activity was measured by the spectrophotometer. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10- 100 µg/ml) was noted after 15 minutes.

1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly (Parkhe G, Jain, 2018). Finally the mean was taken. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm.

$$\text{Calculation of \% Reduction} = \frac{\text{Control Absorbance} - \text{Test absorbance}}{\text{Control Absorbance}} \times 100$$

Antiacne activity of hydroalcoholic extract of *Sterculia lychnophora*:

The well diffusion method was used to determine the antiacne activity of *Sterculia lychnophora* using standard procedure (Bauer, 1966). There were 3

concentration used which are 25, 50 and 100 mg/ml in studies. It's essential feature is the placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted over night broth cultures should never be used as an inoculums. The plates were incubated at 37°C for 24 hr. and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug.

Evaluation of *in vitro* anti-inflammatory activity:

Anti-inflammatory activity of the *Sterculia lychnophora* extract was evaluated by protein denaturation method as described by Padmanabhan and Jangle, (2012). Diclofenac sodium, a powerful

non steroidal anti-inflammatory drug was used as a standard drug. The reaction mixture consisting of 2 mL of different concentrations of *Sterculia lychnophora* extract (100-500 µg/mL) or standard diclofenac sodium (100-500 µg mL⁻¹) and 2.8 mL of phosphate buffered saline (pH 6.4) was mixed with 0.2 mL of egg albumin (from fresh hen's egg) and incubated at (37±1)°C for 15 min. Denaturation was induced by keeping the reaction mixture at 70°C in a water bath for 10 min. After cooling, the absorbance was measured at 660 nm by using double distilled water as blank. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ Inhibition} = \frac{\text{At}-\text{Ac}}{\text{Ac}} \times 100$$

Where, at=absorbance of test sample; Ac=absorbance of control

The plant concentration for 50% inhibition (IC₅₀) was determined by plotting percentage inhibition with respect to control against treatment concentration.

RESULTS AND DISCUSSION:

Table 1 showed the percentage yield of different extract of *Sterculia lychnophora* exhibited percentage yield 0.95% and 6.54% in Petroleum ether and hydroalcoholic extract respectively. Hydroalcoholic extract of *Sterculia lychnophora* exhibited maximum percentage yield followed by Petroleum ether.

The preliminary qualitative analysis of the extract showed the initial information of presence/ absence of the various metabolites in the plant extracts. Table 2 showed the results of phytochemical screening of *Sterculia lychnophora* tannins, flavonoids phenol, proteins, carbohydrates and saponins were detected in hydroalcoholic extracted.

Flavonoids are the largest group of polyphenolic compounds having benzo-γ-pyrone structure and ubiquitous in plants. In this study the quantification of flavanoids in the extract of *Sterculia lychnophora* was determined by aluminum chloride colorimetric method where quercetin was used as a standard. As a type of secondary metabolites, it shows a wide biological activity. The results of total phenol and flavonoids content of *Sterculia lychnophora* were tabulated in table 3.

Table 7.6 shows the results of antioxidant screening test for hydroalcoholic extract of *Sterculia lychnophora* using DPPH method. The comparative radical scavenging effect of Fruits extracts is shown in Figure 4.

The ascorbic acid and extracts have shown dose dependent scavenging of DPPH radicals. The radical scavenging effect of standard and extracts was in the order ascorbic acid > extract IC₅₀ (µg/ml) was found to be 19.29 and 88.37 respectively.

In vitro anti acne activity of *Sterculia lychnophora* hydroalcoholic extract were tested against *Propionibacterium acnes*. After proper incubation the results were recorded and represented in Table 5. These recorded results said that hydroalcoholic extract has high anti acne activity. The present work concluded that *Sterculia lychnophora* has significant secondary metabolites which are responsible for the various pharmacological activities. It also proved that a polar solvent hydroalcoholic is the most effective solvent to extract the metabolites from the *Sterculia lychnophora*.

Table 1: % Yield of hydroalcoholic extract of fruits of *Sterculia lychnophora*

S. No.	Extracts	% Yield (w/w)
1.	Pet. ether	0.95%
2.	Hydroalcoholic	6.54%

Table 2: Phytochemical screening of extract of *Sterculia lychnophora*

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids Mayer's Test Wagner's Test Dragendroff's Test Hager's Test	-ve -ve -ve -ve
2.	Tannins Gelatin Test	+ve
3.	Flavonoids Lead acetate Alkaline test	+ve -ve
4.	Phenol Ferric chloride test	+ve
5.	Proteins Xanthoproteic test	+ve
6.	Carbohydrates Molisch's Test Benedict's Test Fehling's Test	-ve -ve +ve
7.	Saponins Froth Test Foam Test	+ve -ve
8.	Diterpenes Copper acetate test	-ve

Table 3: Estimation of total phenolic and flavonoids content of *Sterculia lychnophora*

S. No.	Hydroalcoholic extract	Total phenol content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1.	<i>Sterculia lychnophora</i>	0.374	0.863

Table 4: % Inhibition of ascorbic acid and hydroalcoholic extract of *Sterculia lychnophora*

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Hydroalcoholic extract
1	10	42.56	19.53
2	20	49.75	26.52
3	40	64.24	31.74
4	60	70.68	39.67
5	80	78.21	47.23
6	100	85.93	53.96
IC₅₀ (µg/ml)		19.29	88.37

Table 5: Antiaene activity of standard drug against *Propionibacterium acnes*

S. No.	Name of drug	Microbes	Zone of inhibition		
			Standard Concentration		
			10 µg/ml	20 µg/ml	30 µg/ml
1.	Clindamycin	<i>Propionibacterium acnes</i>	17±1.69	18±2.62	22±2.16
			Extract Concentration		
2.	Hydroalcoholic extract of <i>Sterculia lychnophora</i>		25mg/ml	50 mg/ml	100mg/ml
			20±0	22.33±4.49	23.66±0.47

Table 6: % Inhibition of Diclofenac sodium and *Sterculia lychnophora* extract

Concentration (µg/ml)	% Inhibition	
	Diclofenac sodium	<i>Sterculia lychnophora</i> extract
100	20.38	17.62
200	45.40	31.54
300	60.04	54.96
400	75.74	65.78
500	90.25	73.62
IC₅₀	250.95	309.36

CONCLUSION:

This is possible to say that the hydroalcoholic extract of *Sterculia lychnophora* contain phytochemical constituents potent and effective. The anti-inflammatory activity of the hydroalcoholic extract of *Eulophia herbacea* might be associated with secondary metabolites. Polyphenols can significantly inhibit a number of inflammatory mediators and prevent the synthesis of prostaglandins.

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