



EVALUATION OF IN-VIVO ANTIACNE ACTIVITY OF MEDICINAL PLANT
CITRULLUS COLOCYNTHIS

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ABSTRACT

Acne is widespread during adolescence, affecting approximately 80-90% of teenagers. Allopathic medications, on the other hand, might have a variety of negative effects. Herbal remedies for acne therapy have been used since ancient times and include diverse herbal extracts, oils, and ayurvedic formulations. *Citrullus colocynthis* medical applications have been described in indigenous systems of medicine from numerous regions. Reviewing this plant usefulness this study deals with evaluation of in-vivo anti-acne activity of medicinal plant *Citrullus colocynthis*. Plant part was gathered, extracted with appropriate solvent investigated for qualitative as well as quantitative analysis. The anti -acne activity of extract was checked in both in vitro & in vivo systems. Results showed that the % Yield of pet. Ether and hydroalcoholic extract of *Citrullus colocynthis* were found to be 3.2% and 8.7% respectively. *Citrullus colocynthis* is a rich source of different secondary metabolites like alkaloids, flavonoids, carbohydrates, saponins and diterpenes. Total flavonoid & alkaloid content of hydroalcoholic extract of *Citrullus colocynthis* was found to be 1.02 mg/ 100 & 0.56 mg/ 100 respectively. The zone of inhibition for *Citrullus colocynthis* extract was observed to be 14±0.57mm, 9±0.94mm & 8±0.5mm at concentration of 100 mg/ml 50 mg/ml & 25mg/ml respectively. The ear thickness was found to be 0.09 at 10th day in clindamycin treated group. In case of *Citrullus colocynthis* extract 100mg/kg & 200mg/kg the ear thickness at 10 th day was observed to be 0.22 & 0.18. Thus, the extract at this dose demonstrated a rapid and sustained reduction in inflammation, highlighting its strong anti-acne potential.

Keywords: Acne, Medicinal plants, *Citrullus colocynthis*, P. acne, Clindamycin, Ear thickness

INTRODUCTION

Acne is a common inflammatory condition that primarily affects adolescents. Seborrheic dermatitis is distinguished by scaly red skin (seborrhea), blackheads and whiteheads (comedones), pinheads (papules), big papules (nodules), pimples, and scarring. Acne affects skin with dense sebaceous follicles, such as

the face, chest, and back. Acne can be inflammatory or non-inflammatory. Androgen stimulation causes lesions due to alterations in pilosebaceous units. Acne is widespread during adolescence, affecting approximately 80-90% of teenagers in the Western world, with a lower rate in rural societies. According to reports, roughly 7.1% of acne patients have suicidal thoughts (Lehmann *et al.*, 2002).

Acne is typically caused by an increase in androgens such as testosterone throughout adolescence in both males and females. Acne fades and eventually disappears as people get older. Large nodules are referred to as cysts, and severe inflammatory acne is referred to as nodulocystic. Cystic acne affects deeper skin tissue than typical acne and appears on the buttocks, groin, armpit area, hair follicles, and perspiration ducts (Beylot et al., 2014; Preneau and Dreno, 2012).

Topical and systemic therapies are used to treat acne. Retinoid is used to treat patients who only have comedones, and it can reduce the quantity of comedones and inflammatory lesions. Others, such as isotretinoin, antibiotics, topical antimicrobials, and hormone treatment, produce excellent results. Allopathic medications, on the other hand, might have a variety of negative effects. When acne-causing bacteria are exposed to antibiotics for an extended period of time, they develop resistance (Cooper and Harris, 2017; Gollnick and Krautheim, 2003).

Herbal remedies for acne therapy have been used since ancient times and include diverse herbal extracts, oils, and ayurvedic formulations. The development of fresh herbal formulations for acne therapy may provide numerous advantages over previously used medicines. These natural antibiotics are efficient against a wide range of Gram-positive and Gram-negative bacteria. The ayurvedic formulation Sunder Vati was shown to be orally effective and well tolerated for the treatment of acne vulgaris. Purintablets and klarina cream formulations, which include numerous plant extracts and have low side effects when compared to contemporary

treatment, are often used to treat moderate to severe acne (Yarnell and Abascal et al., 2006; Nasri et al., 2015).

Citrullus colocynthis (L.) Schrad is a desirable plant from the Cucurbitaceae family that is widely spread in the barren region. It is a non-hardy, herbaceous perennial vine that is branching from the base. The medical applications of this plant have been described in indigenous systems of medicine from numerous regions, including stomach problems such as indigestion, colic, gastroenteritis, and dysentery. Plants are traditionally used to treat diabetes in equatorial and subequatorial nations. It is also used to treat high blood pressure in Morocco. The fruits are used to treat bacterial infections, digestive diseases, diabetes, and cancer in India, Bangladesh, Nepal, and Pakistan. Because of its anti-inflammatory properties, it is one of the most popular inhabitant medicines in the UAE. In Mediterranean regions, the plant's fruits and seeds are used to treat UTI as well as other disorders such as rheumatism, hypertension, pulmonary difficulties, dermatological problems, and gynaecological infections. Fruits are used as an anthelmintic, antirheumatic, purgative, carminative, and laxative in Saudi Arabia and Israel (Pravin et al., 2013; Rahimi et al., 2012; Rao and Poonia, 2023). Reviewing this plant usefulness this study deals with evaluation of in-vivo anti-acne activity of medicinal plant *Citrullus colocynthis*.

MATERIALS & METHODS

Collection of plant: The fruit of *Citrullus colocynthis* were collected from local area of Bhopal in the month of February, 2023.

Defatting & extraction

48 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. Defatted powdered of *Citrullus colocynthis* has been extracted with hydroalcoholic solvent (Ethanol: water; 80:20v/v) using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40°C

Determination of percentage yield

The % yield was calculated by dividing weight of extract by weight of powdered drug.

Phytochemical screening

Phytochemical examinations were carried out for all the extracts as per the standard methods (Pandey and Tripathi, 2014).

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method (Shraim et al., 2021). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filtered. 3 ml (1mg/ml) of this solution was used for the estimation of flavonoid. 1 ml of 2% AlCl₃ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

Estimation of total alkaloids content

The plant extract (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered (Ajanal et al., 2012). 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 (40, 60, 80, 100 and 120 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/100mg of extract.

In vitro antimicrobial activity

Agar well diffusion method was used for this purpose. *P.acne* were used for the study. The media was poured into sterilized petri dishes, and the media was stand still and allowed to solidify. The bacterial cultures were spread properly. Then, with the help of sterile cork borer wells were made in the petri dishes of 6mm diameter each, to which the prepared formulations were added and allowed the drug to spread in the media. Then it was incubated for 24 h at 37 °C. The diameter of the zone of inhibitions was observed and, with the help of a ruler, was measured (in mm). Each formulation's antibacterial activity was measured in triplicate form, and their mean value was recorded.

Animals

Wistar rats (180-220g) were group housed (n=6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2°C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory

conditions for 7 days before carrying out the experiments.

Acute toxicity studies

Acute oral toxicity was conducted according to the method of Organisation for Economic Co-operation and Development (OECD) (OECD, 2001). Animals were kept fasting providing only water, hydroalcoholic extract of *Citrullus colocynthis* (250, 500, 1000, 2000mg/kg/day) was administered orally for 4 days of five groups of rats (n=6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation of a possible anti-acne activity.

Experimental designs

Group -I: control (acne induced)

Group -II: Hydroalcoholic extract of *Citrullus colocynthis* (100mg/kg, p.o.)

Group -III: Hydroalcoholic extract of *Citrullus colocynthis* (200mg/kg, p.o.)

Group -IV: Clindamycin (200mg/kg, p.o.)

Measurement of ear thickness

Ear thickness was measured as an index of inflammatory strength and acne. Thickness was measured by using a vernier calliper. Thickness was measured once every two day until the 10th day.

Statistical analysis

All statistical analysis is expressed as mean \pm standard error of the mean (SEM). Data were analyzed by one way ANOVA, where applicable $p < 0.05$ was considered statistically significant, compared with vehicle followed by Dunnett's test.

RESULTS AND DISCUSSION

The % Yield of pet. ether and hydroalcoholic extract of *Citrullus colocynthis* were found to be 3.2% and 8.7% respectively. Qualitative phytochemical analysis clearly demonstrated the presence of number important active constituents and revealed that *Citrullus colocynthis* have similar phytochemical constitution. The results revealed that the plant is a rich source of different secondary metabolites like alkaloids, flavonoids, carbohydrates, saponins and diterpenes. The assorted phytochemicals are common compounds to give pharmacological benefit.

Total flavonoid & alkaloid content of hydroalcoholic extract of *Citrullus colocynthis* was found to be 1.02 mg/ 100 & 0.56 mg/ 100 respectively.

The antimicrobial activity of *Citrullus colocynthis* was checked by agar well diffusion method. The standard drug clindamycin used as a reference. The zone of inhibition for clindamycin against acne was found to be 17 ± 0.74 mm, 13 ± 0.5 mm & 10 ± 0.57 mm for 30 μ g/ml, 20 μ g/ml & 10 μ g/ml respectively. Further the zone of inhibition for *Citrullus colocynthis* extract was observed to be 14 ± 0.57 mm, 9 ± 0.94 mm & 8 ± 0.5 mm at concentration of 100 mg/ml 50 mg/ml & 25mg/ml respectively.

The results of the study indicate that both the standard drug Clindamycin and the hydroalcoholic extract possess antiacne activity against *Propionibacterium acnes*. The zone of inhibition observed for the hydroalcoholic extract suggests its potential as an alternative or complementary treatment for acne. While Clindamycin exhibited slightly higher inhibitory effects at the highest

concentration tested, the hydroalcoholic extract demonstrated significant inhibitory activity as well.

The ear thickness was found to be 0.09 at 10th day in clindamycin treated group. In case of *Citrullus colocynthis* extract 100mg/kg & 200mg/kg the ear thickness at 10th day was observed to be 0.22 & 0.18. The ear thickness measurements in this group were significantly

lower compared to both the control group and the lower dose extract-treated group at all time points. The extract at this dose demonstrated a rapid and sustained reduction in inflammation, highlighting its strong anti-acne potential.

Table 1: % Yield of hydroalcoholic extract of *Citrullus colocynthis*

S. No.	Extracts	% Yield (w/w)
1.	Pet. ether	3.2%
2.	Ethanollic	8.7%

Table 2: Phytochemical screening of fruit extract of *Citrullus colocynthis*

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids Mayer's Test Wagner's Test Dragendroff's Test Hager's Test	-ve -ve +ve +ve
2.	Glycosides Legal's 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	+ve
8.	Diterpenes Copper acetate test	+ve
9.	Tannins Gelatin Test	-ve

Table 3: Estimation of total flavonoids and alkaloid content of fruit extract of *Citrullus colocynthis*

S. No.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)	Total alkaloid content (mg/ 100 mg of dried extract)
1.	Hydroalcoholic	1.02	0.56

Table 4: Antiacne activity of standard drug and hydroalcoholic extract against *Propionibacterium acnes*

S. No.	Drug	Zone of Inhibition (nm)		
		30 µg/ml	20 µg/ml	10 µg/ml
1	Clindamycin	17±0.74	13±0.5	10±0.57
		100 mg/ml	50 mg/ml	25mg/ml
2	Hydroalcoholic extract	14±0.57	9±0.94	8±0.5

Table 7.3: Effect of Clindamycin (standard) and hydroalcoholic extract of *Citrullus colocynthis* on acne induced by *Propionibacterium acnes* in rats

Treatment	Dose	Mean thickness ±SEM				
		Day 2	Day 4	Day 6	Day 8	Day 10
Control	140 µg	1.49	1.35	1.28	1.25	1.24
<i>Citrullus colocynthis</i> extract	100mg/kg p.o.	1.45	0.35	0.22	0.22	0.22
<i>Citrullus colocynthis</i> extract	200mg/kg p.o.	1.15	0.26	0.2	0.18	0.18
Clindamycin	200 mg/kg p.o.	0.98	0.18	0.1	0.09	0.09

CONCLUSION

The study clearly demonstrates that the hydroalcoholic extract of *Citrullus colocynthis* possesses potent anti-acne activity in the rat ear inflammatory model induced by *Propionibacterium acnes*. Both doses of the extract (100mg/kg and 200mg/kg) exhibited a dose-dependent reduction in ear thickness, reflecting their ability to counteract the inflammatory response. The anti-acne effect of *Citrullus colocynthis* extract was comparable to that of the standard drug

Clindamycin, indicating its promising potential as an alternative treatment for acne-associated inflammation. This anti acne potential may be attributed to phytochemicals present in this plant. Further clinical trials are needed to prove its efficacy & safety in treating acne.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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