



EVALUATION OF IN-VIVO ANTIACNE PROPERTIES OF CITRULLUS COLOCYNTHIS, A MEDICINAL PLANT

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Abstract

About 80–90% of teens experience acne at some point during their youth. Conversely, allopathic drugs may have a number of unfavourable side effects. Various botanical extracts, oils, and ayurvedic formulations have been employed as natural therapies for acne therapy since ancient times. Numerous indigenous medical systems around the world have documented the use of *Citrullus colocynthis* in medicine. Examining the plant's use, this study assesses *Citrullus colocynthis*, a medicinal herb, for its in-vivo anti-acne properties. Plant portion was harvested, extracted with appropriate solvent analysed for qualitative as well as quantitative examination. The extract's anti-acne properties were examined in both in vitro and in vivo systems. The percentage yield of *Citrullus colocynthis*'s pet ether and hydroalcoholic extract was determined to be 3.2% and 8.7%, respectively, according to the results. Numerous secondary metabolites, including alkaloids, flavonoids, carbohydrates, saponins, and diterpenes, are abundant in *Citrullus colocynthis*. *Citrullus colocynthis* hydroalcoholic extract was found to have a total flavonoid and alkaloid concentration of 1.02 mg/100 and 0.56 mg/100, respectively. *Citrullus colocynthis* extract was shown to have a zone of inhibition measuring 14 ± 0.57 mm, 9 ± 0.94 mm, and 8 ± 0.5 mm at concentrations of 100 mg/ml, 50 mg/ml, and 25 mg/ml, in that order. On day ten, the ear thickness in the group receiving clindamycin was determined to be 0.09. After 10 days, the ear thickness values for *Citrullus colocynthis* extract at 100 mg/kg and 200 mg/kg were found to be 0.22 and 0.18, respectively. As a result, the extract at this dosage showed a robust anti-acne effect by reducing inflammation quickly and persistently.

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

Introduction

A frequent inflammatory illness that mostly affects teenagers is acne. The telltale signs of seborrheic dermatitis include scaly red skin (seborrhea), comedones (black and white pimples), papules (pinheads), large papules (nodules), acne, and scarring. Skin on the face, chest, and back with thick sebaceous follicles is susceptible to acne. Both inflammatory and non-inflammatory acne can occur. Because androgen stimulation alters pilosebaceous units, lesions result. In the West, 80–90% of teenagers suffer from acne during adolescence; in rural societies, the prevalence is lower. Roughly 7.1% of acne sufferers report having suicidal thoughts (Lehmann et al., 2002).

Acne is often caused by an increase in androgens such as testosterone throughout puberty in both boys and girls. As people age, acne gradually lessens and goes away. Severe inflammatory acne is called nodulocystic, and large nodules are called cysts. Unlike regular acne, cystic acne affects deeper layers of skin tissue and can be seen on the groyne, armpit area, hair follicles, and sweat ducts (Beylot et al., 2014; Preneau and Dreno, 2012).

Acne is treated with both topical and systemic treatments. Patients with comedones alone can benefit from retinoid therapy, which can lessen the number of inflammatory lesions and comedones. Other treatments that work well include hormone therapy, topical antimicrobials, antibiotics, and isotretinoin. Conversely, allopathic drugs may have a number of unfavourable side effects. Antibiotic-resistant bacteria are those that cause acne when they are exposed to them over an extended length of time (Cooper and Harris, 2017; Gollnick and Krautheim, 2003).

Various botanical extracts, oils, and ayurvedic formulations have been employed as natural therapies for acne therapy since ancient times. The creation of novel herbal remedies for the treatment of acne may have many benefits over the usage of older medications. The effectiveness of these natural antibiotics against a variety of Gram-positive and Gram-negative bacteria is high. It has been demonstrated that the ayurvedic formulation Sunder Vati is a well-tolerated oral therapy for acne vulgaris. When treating

moderate to severe acne, purintablets and klarina cream formulations are frequently utilised because they contain a variety of plant extracts and have fewer adverse effects than modern treatments (Yarnell and Abascal et al., 2006; Nasri et al., 2015).

A popular plant in the arid area, *Citrullus colocynthis* (L.) Schrad is a member of the Cucurbitaceae family. This perennial vine is herbaceous, non-hardy, and branches from the root. Indigenous medical systems from several locations have documented the use of this herb for treating stomach ailments such as dysentery, colic, indigestion, and gastroenteritis. Traditionally, equatorial and subequatorial countries have treated diabetes with plants. In Morocco, it's also used to treat excessive blood pressure. In India, Bangladesh, Nepal, and Pakistan, the fruits are used to cure cancer, diabetes, gastrointestinal disorders, and bacterial infections. It is one of the most widely used resident medications in the United Arab Emirates due to its anti-inflammatory qualities. The fruits and seeds of the plant are used in Mediterranean regions to treat rheumatism, hypertension, lung issues, dermatological conditions, and gynaecological diseases in addition to urinary tract infections. In Israel and Saudi Arabia, fruits are used as a laxative, anthelmintic, carminative, purgative, and antirheumatic (Pravin et al., 2013; Rahimi et al., 2012; Rao and Poonia, 2023). Examining the plant's use, this study assesses *Citrullus colocynthis*, a medicinal herb, for its in-vivo anti-acne properties.

Materials & Methods

Collection of plant

In February 2023, the fruit of *Citrullus colocynthis* was harvested from the nearby Bhopal area.

Defatting & extraction

48 gram sample of shade-dried plant material was ground into a coarse powder and macerated in petroleum ether to remove the substance. Until the material had undergone defatting, the extraction process was maintained. After being macerated for 48 hours to extract the defatted powdered *Citrullus colocynthis*, the hydroalcoholic solvent (ethanol: water; 80:20v/v) was filtered and dried at 40°C in a vacuum evaporator.

Phytochemical screening

According to conventional procedures, phytochemical analyses were performed on each extract (Pandey and Tripathi, 2014).

Estimation of total flavonoids content

The aluminium chloride method was used to determine the total flavonoid concentration (Shraim et al., 2021). 10 ml of methanol were used to dissolve 10 mg of quercetin, and different aliquots containing 5–25 µg/ml were made. Using 10 millilitres of methanol, 10 mg of dried extracts were dissolved and filtered. The flavonoid was estimated using 3 millilitres (1 milligramme per millilitre) of this solution. After adding 1 ml of a 2% AlCl₃ methanolic solution to 3 ml of extract or standard, the mixture was left to stand at room temperature for 15 minutes. The absorbance was then measured at 420 nm.

Estimation of total alkaloids content

After dissolving 1 mg of the plant extract in methanol and adding 1 ml of 2 N HCl, the mixture was filtered (Ajanal et al., 2012). After this solution was moved to a separating funnel, five millilitres each of phosphate buffer and bromocresol green solution were added. The mixture was collected in a 10-ml volumetric flask and diluted to the volume with chloroform after being vigorously agitated with 1, 2, 3, and 4 ml of the chloroform. In the same way as previously mentioned, a series of reference standard solutions containing 40, 60, 80, 100, and 120 µg/ml of atropine were created. Using a UV/Visible spectrophotometer, the absorbance of the test and standard solutions was measured at 470 nm in relation to the reagent blank. The alkaloid concentration was measured in milligrammes of AE per 100 milligrams of extract.

In Vitro Antimicrobial activity

For this, the Agar well diffusion method was employed. *P. acne* was employed in the research. Sterilised petri dishes were filled with the medium, which was then left to stand still and harden. The cultures of bacteria were dispersed appropriately. Subsequently, 6 mm diameter wells were created in the petri dishes using sterilised cork borer, to which the produced formulations were poured, enabling the medicine to diffuse throughout the medium. It was then incubated at 37 °C for 24 hours. Using a ruler, the diameter of the

zone of inhibitions was measured (in millimetres). The antibacterial activity of each formulation was evaluated in triplicate, and the average result was noted.

Animals

Wistar rats weighing between 180 and 220 g were kept in groups of six in controlled temperatures and humidity levels of $25\pm 2^{\circ}\text{C}$ and 55–65% respectively, with a regular 12-hour light/dark cycle. Water was available at all times, along with conventional rat feed. Prior to doing the trials, the rats were given seven days to become used to the lab environment.

Acute toxicity studies

The Organisation for Economic Co-operation and Development (OECD) protocol for acute oral toxicity was followed (OECD, 2001). The rats ($n = 6$) in five groups were given an oral hydroalcoholic extract of *Citrullus colocynthis* (250, 500, 1000, and 2000 mg/kg/day) for four days while they were fasted and monitored for behavioural changes and animal mortality to assess any potential 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

An indicator of acne and inflammatory intensity was ear thickness. A vernier calliper was used to measure thickness. Up to the tenth day, thickness was measured once every two days.

Analytical statistics

The standard error of the mean (SEM) is represented as the mean $\pm$ in all statistical analyses. Data were compared with vehicles using Dunnett's test after one way ANOVA analysis, when appropriate $p < 0.05$ was deemed statistically significant.

Results & Discussion

The % Yield of pet. ether and hydroalcoholic extract of *Citrullus colocynthis* were determined to be 3.2% and 8.7% respectively. A qualitative phytochemical examination showed that *Citrullus colocynthis* has a comparable phytochemical constitution and amply indicated the existence of several significant active components. The plant is a rich source of many secondary metabolites, including alkaloids, flavonoids, carbohydrates, saponins, and diterpenes, according to the results. The many phytochemicals are typical substances that have pharmacological advantages.

Citrullus colocynthis hydroalcoholic extract was found to have a total flavonoid and alkaloid concentration of 1.02 mg/100 and 0.56 mg/100, respectively.

Using the agar well diffusion method, *Citrullus colocynthis*' antibacterial activity was examined. Clindamycin, a common medication, was utilised as a benchmark. It was discovered that the clindamycin zone of inhibition against acne was 17 ± 0.74 mm, 13 ± 0.5 mm, and 10 ± 0.57 mm for 30 μ g/ml, 20 μ g/ml, and 10 μ g/ml, respectively. Additionally, the extract of *Citrullus colocynthis* was shown to have a zone of inhibition measuring 14 ± 0.57 mm, 9 ± 0.94 mm, and 8 ± 0.5 mm at concentrations of 100 mg/ml, 50 mg/ml, and 25 mg/ml, respectively.

The study's findings show that *Propionibacterium acnes* can be inhibited by the hydroalcoholic extract and the prescription medication Clindamycin. The hydroalcoholic extract's zone of inhibition indicates that it may be used in addition to or as a substitute treatment for acne. At the highest concentration tested, clindamycin showed somewhat stronger inhibitory effects, although the hydroalcoholic extract also showed a notable amount of inhibitory activity.

On day ten, the ear thickness in the group receiving clindamycin was determined to be 0.09. After 10 days, the ear thickness values for *Citrullus colocynthis* extract at 100 mg/kg and 200 mg/kg were found to be 0.22 and 0.18, respectively. At every time point, this group's ear thickness measures were considerably smaller than those of the lower dose extract-treated group and the control group. At this dosage, the extract showed a robust anti-acne effect by reducing inflammation quickly and persistently.

Table 1: 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 -+e +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 2: 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 3: 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 4: Effect of Clindamycin (standard) and hydroalcoholic extract of *Citrullus colocynthis* on acne induced by *Propionibacterium acnes* in rats

Treatment	Dose	Mean thickness $\pm$ SEM				
		Day2	Day4	Day6	Day8	Day10
Control	140 μ g	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

This study reveals that *Citrullus colocynthis* hydroalcoholic extract has strong anti-acne properties in the rat ear inflammation model caused by *Propionibacterium acnes*. The extract's capacity to inhibit the inflammatory response was demonstrated by the dose-dependent decrease in ear thickness that both the 100 mg/kg and 200 mg/kg doses showed. *Citrullus colocynthis* extract showed a comparable anti-acne impact to the usual medication Clindamycin, suggesting that it has promising potential as a substitute treatment for inflammation linked to acne. This plant's phytochemical content may be responsible for its anti-acne properties. Additional clinical trials are required to demonstrate its safety & effectiveness in treating acne.

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