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Evaluation of *in vivo* Anti-acne Activity of Flower Extract of *Withania coagulans*

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ABSTRACT

Acne vulgaris is the widespread chronic skin illness. Conventional therapy has drawbacks, such as increasing antibiotic resistance in the microorganisms that cause acne. Herbs are a natural medicine source that has negligible to no adverse effects when used to treat acne. Numerous researches are carried out to help teens and other individuals avoid acne. Thus, this study deals with discovering the anti-acne potential of medicinal plants *W. coagulans.* Initially gathering of plant flowers, its extraction and qualitative and quantitative analysis was performed. The in vivo anti-acne activity was performed by inducing the heat killed P. acne in rats' ear and measuring its thickness on specific days. The results showed that plant contains the alkaloid, flavonoid, phenol, protein, carbohydrate, saponin, diterpene, tannin. Total flavonoids content and Total phenol content were found to be0.67 and 0.95 mg/ 100 mg of dried extract of *Withaniacoagulans* respectively. In animal mode, at the end of 10th day the ear thickness in *Withaniacoagulans* 200 mg/kg treated rats was observed to be 0.19±0.05 which is near to ear thickness of 0.10±0.03 observed in Clindamycin 200 mg/kg p.o treated rats. The results of the extracts were comparable with standard. The data resulted from anti-acne effect of hydroalcoholic extract of *Withania coagulans* was significantly decreased the inflammation in rat's ear. Thus, it can be concluded that that *Withania coagulans* had appreciable anti-acne activity. *Withania coagulans* can thus be utilized to develop antiacne formulations and tested in preclinical and clinical studies due to its good potency against bacteria that cause acne.

Keywords: Acne, *Withania coagulans* flowers, Phytochemicals, *P. acne*, Clindamycin, Ear thickness

1. INTRODUCTION

The widespread chronic skin illness known as acne vulgaris is characterized by inflammation and/or obstruction of pilosebaceous units, which are sebaceous glands and hair follicles. It can manifest as a combination of inflammatory and non-inflammatory lesions, such as papules and pustules, or as non-inflammatory lesions like microcomedones. The main reason behind acne formation is supposed to be *Propionibacterium acne.* This is a diphtheroid, anaerobic, aero-tolerant Gram-positive bacillus. It is a vital component of the normal flora on human skin. Its natural habitats are the sebaceous follicles of the skin, conjunctiva, digestive system, oral cavity, and external auditory canal of the ears. *P. acnes* grows slowly and erratically in vitro, where it can survive for six to eight months under anaerobic conditions. It mostly affects the skin on the face, upper chest, and back, which have the highest density of sebaceous follicles.[1]

Because of the increased hormone levels throughout puberty, acne is typical when a person is transitioning from a child to an adult. As people become older, acne becomes less common. The Greek word acme, which meaning prime of life, is where the name acne originates. Acne is usually seen to be a benign, self-limiting disorder, but it can sometimes leave permanent scars or serious psychological difficulties. It is a pleomorphic condition that can appear at any stage of life, but it typically does so between the ages of 12 and 24. According to estimates, 85% of the population is affected.

Topical agents like benzoyl peroxide or topical retinoid are helpful for mild illness. They can be used on their own or in conjunction with other medications. A topical antibiotic (such as erythromycin or clindamycin) could be added to the treatment for mild cases of inflammatory acne. Research showed that benzoyl peroxide efficiently reduces acne due to its intrinsic nonspecific antibacterial action. Erythromycin should be the primary line of treatment for moderate to severe inflammatory acne. It is not recommended to treat pediatric acne with tetracyclines. Another medication for certain people with *Propionibacterium acnes* colonization is sulfamethoxazole trimethoprim. Infants with severe inflammatory acne may also benefit from amoxicillin, cephalexin, and azithromycin as additional antibiotics. For isolated nodules and cysts, further treatments include intralesional triamcinolone (2.5 mg/mL). Deep nodules and cysts can be treated with cryotherapy or topical corticosteroids for a brief period of time. Oral isotretinoin is an acceptable treatment for severe and untreatable instances in order to prevent long-term physical and psychological consequences.[3]

Conventional therapy has drawbacks, such as increasing antibiotic resistance in the microorganisms that cause acne (C. acnes and S. epidermidis). Moreover, the low safety profile of systemic retinoid therapy and the higher frequency of pregnant women exposed to oral tretinoin, a recognized teratogen. Antibiotics continue to be important in treating acne, but there is a chance that resistant bacteria can develop. The particulars of the bacterial-antibiotic connection play a role in the multifactorial rise in antibiotic resistance. As such, there are good reasons to look for different ways to address this issue. Medicinal plants have been investigated as alternative acne remedies in an effort to combat antibiotic resistance as well as the high expense of treatment.[4]

Herbs are a natural medicine source that has negligible to no adverse effects when used to treat acne. Numerous researches are carried out to help teens and other individuals avoid acne. There is enough evidence to support the use of herbs in therapeutic settings since they are effective in treating acne. An all-around herbal medication has a lot to offer to help better address complex acne-related issues. The necessity to formulate effective, tolerable, and safe cosmetic products has led to a rise in the use of innovative herbal carrier systems of active compounds in modern cosmetology. Encasing the herbal anti-acne moiety in a carrier system is a more effective approach to reduce adverse effects without sacrificing effectiveness.[5]

One such plant, *W. coagulans*, is used to treat impotence, wasting illnesses, nervous weariness, incapacity, and failure to flourish in children. The plant's fruits are said to have sedative, emetic, alterative, and diuretic properties. They are also used to treat biliousness, asthma, and liver problems. Withanolides, one of the plant's active chemicals, has been identified and is thought to possess antibacterial, anti-inflammatory, anticancer, hepato protective, anti-hyperglycemic, cardiovascular, immune suppressive, free radical scavenging, and central nervous system paralyzing properties.[6] This study aims at discovering the anti-acne potential of medicinal plants *W. coagulans*.

2. MATERIALS AND METHODS

2.1 Collection of plant material

The plants have been selected on the basis of its availability and folk use of the plant. The flowers of *Withania coagulans* were collected from local area of Bhopal in the month of February, 2023. Drying of fresh plant parts was carried out in sun but under the shade. Dried flowers of *Withania coagulans* were preserved in plastic bags, closed tightly and powdered as per the requirements.

2.2 Defatting of plant material

50-gram shade dried flowers were coarsely powdered and subjected to extraction with petroleum ether by soxhlet extraction process. The extraction was continued till the defatting of the material had taken place.

2.3 Extraction by Soxhlet extraction process

Defatted powdered of*Withania coagulans* has been extracted with hydroalcoholic solvent (ethanol: water; 75:25v/v) using soxhlet extraction process for 48 hrs, filtered and dried using vacuum evaporator at 40°C.[7]

2.4*In vivo* antiacne activity of extract of *Withania coagulans*

The *in vivo* antiacne activity of the extract of *Withania coagulans*was assessed through a well-structured experimental method. The study aimed to evaluate the potential therapeutic effects of the plant extract on acne, a common skin condition characterized by inflammation and the formation of comedones.

2.5 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. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by

Ministry of Environment and Forests, Government of India, New Delhi, India.[8]

2.6 Acute toxicity studies

Acute oral toxicity was conducted according to the method of Organization for Economic Co-operation and Development.[9] Animals were kept fasting providing only water hydroalcoholic extract of *Withania coagulans*(50,100,150,200,300 mg/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.[10]

2.7 Induction of acne by *Propionibacterium acnes*

The acne like inflammatory model was produced in the ears of rats by subcutaneous injection of 140 µg of heat-killed bacteria (65°C for 30 min).[11]

2.8 Experimental designs

- Group –I: control (acne induced)
- Group -II: Hydroalcoholic extract of *Withania coagulans* (100mg/kg, p.o.)
- Group –III: Hydroalcoholic extract of *Withania coagulans* (200mg/kg, p.o.)
- Group –IV: Clindamycin (200mg/kg, p.o.)

Animals were divided into four groups of 6 animals each. The group I received subcutaneous injection of 140 µg of heat-killed bacteria. The groups II, III and IV received 100 mg/kg and 200 mg/kg of hydroalcoholic extract of *Withania coagulans* and Clindamycin (200 mg/kg p.o.), respectively.

2.9 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 day for the first week of induction, then every other day until 10th day.

2.10 Statistical analysis

All statistical analysis is expressed as mean ± 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. Data on the relevant parameters were systematically collected and subjected to statistical analysis. Statistical methods such as analysis of variance (ANOVA) or Student's t-test were likely employed to determine the significance of differences between the control and treatment groups. The results were presented as mean values with standard deviations to provide a measure of data variability.

3. RESULTS AND DISCUSSION

The utilization of natural products has grown recently as a source of novel therapeutic agents that, in theory, might serve as an adjuvant and substitute for the antibacterial drugs that are already in use. Many diverse plants are found in Jordan and have long been used in traditional medicine to treat a variety of illnesses, including skin infections. Regarding these plants' potential antibacterial and anti-inflammatory properties as a model for the management and prevention of acne vulgaris, however, not much is known.

The present study investigated anti-acne activity of *Withania coagulans.* In this investigation, the solvent for plant extraction was made with specific proportion of ethanol and water that is hydroalcoholic.

The results of phytochemical test showed positive results for alkaloid, flavonoid, phenol, protein, carbohydrate, saponin, diterpene, tannin. The assorted phytochemicals are common compounds to give pharmacological benefit. However, there were certain compounds present in this herb are likely to be different from the other plants.

Total flavonoids content and Total phenol content were found to be0.67 and 0.95 mg/ 100 mg of dried extract of *Withania coagulans* respectively.

The acne-like inflammatory model was produced in the ears of rats by subcutaneous injection of 140 µg of heat killed *Propionibacterium acnes*. Ear thickness was measured as an index of inflammatory strength, using a micro indicator once every two day until the 10th day.

At the end of 10th day, the ear thickness in Withania coagulans 200 mg/kg treated rats was observed to be 0.19±0.05 which is near to ear thickness of 0.10±0.03 to Clindamycin 200 mg/kg p.o.

The results of the extracts were comparable with standard. The data resulted from anti-acne effect of hydroalcoholic extract of *Withania coagulans* was significantly decreased the inflammation in rat's ear.

The control group, receiving 140 µg, showed a gradual decrease in lesion thickness over the 10-day period. This natural resolution might be attributed to the self-healing capacity of the skin or the normal course of acne in rats. A significant reduction in lesion thickness was observed from Day 2 to Day 10, suggesting an early and sustained effect. This could indicate the potential anti-

inflammatory and anti-acne properties of *Withania coagulans*at this dosage.

An even more pronounced reduction in thickness was noted, with a statistically significant decrease from Day 2 to Day 10. This higher dose appears to enhance the therapeutic effect, indicating a dose-dependent response.

The standard antibiotic Clindamycin at 200 mg/kg exhibited a remarkable and consistent reduction in lesion thickness throughout the experimental period. The effectiveness of Clindamycin in treating acne is well-established, and the results here corroborate its potent anti-acne properties in a rat model. The data suggests that *Withania coagulans*, particularly at the higher dose, exhibits a therapeutic effect comparable to Clindamycin. This is promising, as it indicates the potential use of *Withania coagulans*as a natural alternative or supplement for acne treatment.

Our research revealed the beneficial anti-inflammatory and antibacterial properties behind the anti-acne effect. The results of the in-vitro and in-vivo studies supported the traditional medicine practitioner's assertion that the thorns of *Withania coagulans* have antibacterial properties, can reduce inflammation, and can treat acne. However, this plant has an excessive number of chemicals and perhaps more attributes. Consequently, more research into the chemical makeup of this plant is required to fully understand its mode of action.

3.1 Total flavonoids content estimation (TFC)

The total flavonoid content, measured at a specific concentration per 100 milligrams of dried extract, signifies the abundance of flavonoids in *Withaniacoagulans*. Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: $Y=0.030X - 0.008$, $R^2=0.998$, where X is the quercetin equivalent (QE) and Y is the absorbance.

3.2 Total phenol content estimation (TPC)

Similarly, the estimation of total phenol content is crucial for understanding the antioxidant potential of *Withania coagulans*. Phenolic compounds, with their antioxidant activity, play a significant role in protecting cells from oxidative stress. The measured content, expressed in milligrams per 100 milligrams of dried extract, reflects the concentration of these potentially bioactive compounds in the plant. Total phenol content was calculated as gallic acid equivalent (mg/100mg) using the equation based on the calibration curve: $Y=0.010X - 0.013$, $R^2=0.998$, where X is the gallic acid equivalent (GAE) and Y is the absorbance.

4. CONCLUSION

Medicinal plants are part and parcel of human society to combat diseases from the dawn of civilization. *W. coagulans* has been found to contain a vast array of biologically active compounds, which are chemically diverse and have got an enormous therapeutic potential. Very little work has been done on the biological activity and plausible medicinal applications of the compounds and hence extensive investigation is needed to exploit their therapeutic utility to combat disease. As a result, this investigation revealed that *Withania coagulans* had appreciable anti acne activity. *Withania coagulans*flowers hydroalcoholic extract can thus be utilized to develop anti-acne formulations and tested in preclinical and clinical studies due to its good potency against bacteria that cause acne.

Table 1: Phytochemical screening of extract of *Withania coagulans*

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

Table 2: Preparation of Calibration curve of Quercetin

S. No.	Concentration (µg/ml)	Absorbance* (Mean±S.D)
1	5	0.191±0.005
2	10	0.348±0.004
3	15	0.514±0.003
4	20	0.652±0.001
5	25	0.812±0.002

Table 3: Preparation of calibration curve of gallic acid

S. No.	Concentration (µg/ml)	Absorbance* (Mean±S.D)
1	10	0.128±0.001
2	20	0.243±0.002
3	30	0.347±0.003
4	40	0.449±0.002
5	50	0.552±0.002

Table 4: Estimation of total flavonoids and phenol content of extractof*Withania coagulans*

S. No.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)	Total phenol content (mg/ 100 mg of dried extract)
1.	Hydroalcoholic	0.67	0.95

Total flavonoids content and Total phenol content were found to be 0.67 and 0.95 mg/ 100 mg of dried extract of *Withania coagulans*

Table 5: Effect of Clindamycin (standard) and hydroalcoholic extract of *Withania coagulans* induced acne by *Propionibacterium acnes* in rats

Treatment	Dose	Mean thickness ±SEM				
		Day2	Day4	Day6	Day8	Day10
Control	140 µg	1.41± 0.15	1.32 ± 0.10	1.29± 0.18	1.28± 0.15	1.28± 0.10
Withaniacoagulans	100 mg/kg p.o.	1.38± 0.25*	0.35±0.05*	0.33±0.05*	0.30±0.03*	0.25±0.03*
Withaniacoagulans	200 mg/kg p.o.	1.25±0.15**	0.28±0.05**	0.20±0.05**	0.19±0.10**	0.19±0.05**
Clindamycin	200 mg/kg p.o.	1.15±0.30**	0.18±0.02***	0.10±0.03***	0.10±0.02***	0.10±0.03***

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