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PHYTOCHEMICAL AND ANTIMICROBIAL SCREENING OF *ENTADA PHASEOLOIDES* SEED EXTRACT FOR THE TREATMENT OF ACNE

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ABSTRACT
The most prevalent skin condition affecting adolescents is acne vulgaris. Seborrheic skin, comedones and blackheads, papules, pimples, and scars are the hallmarks of acne vulgaris. These symptoms can cause loss of self-esteem leading to depression. There are many different synthetic drug treatments plans available for acne vulgaris but none of these regimens is without risks. Compared to modern therapies, herbal cures typically have fewer adverse effects. *E. phaseoloides* a member of the Fabaceae family has a long history of use in Indian traditional medicine, where it is used to treat a variety of conditions including brain haemorrhage and as an emetic, anti-irritant, promoter of hair growth, and painkiller. This study deals with analysing anti acne potential of seeds of *E. phaseoloides.* Plant material was collected & subjected to extraction. All other In vitro & In vivo procedures are then performed according to standard protocol. Results revealed that yield was found to be (6.47% w/w of crude drug) of hydroalcoholic extract. The phytochemical screening of *Entada phaseoloides* showed the presence of various classes of secondary metabolites, including alkaloids, flavonoids, saponins, tannins, terpenoids, and glycosides. The total phenolic & flavonoid content was found to be 0.536mg GAE/100mg of dry weight & 0.764mg QE/100mg of dry weight. The zone of inhibition for *P.acne* was found to be 18±0.57mm for 100mg/ml of dried extract. While for *E. coli* it was found to be 16±0.74mm. In case of *A. flavus* the zone of inhibition was found to be 8±0.86mm. Rats showed that both Clindamycin and hydroalcoholic extract of *Entada phaseoloides* were effective in reducing the severity of acne induced by Propionibacterium acnes. However, Clindamycin was found to be more effective in reducing the number of inflammatory cells in the affected skin, while *Entada phaseoloides* extract was found to be more effective in reducing the size of the lesions. From the results it can be concluded that seeds of *E. phaseoloides* possess appreciable anti acne activity.

Keywords: Medicinal plants, Herbal medicines Anti acne, Acne vulgaris, *Entada phaseoloides, P.acne*.

INTRODUCTION

Acne vulgaris (AV) is a persistent skin disorder that causes the pilosebaceous glands to become inflamed. Not just teenagers but also adults are affected by acne. According to the Global Burden of Disease (GBD) study, AV affects roughly 85% of young adults between the ages of 12 and 25. It is known that AV symptoms influence the development of depression, which lowers the quality of life for

its sufferers, particularly adolescents. AV has been linked to psychological comorbidities such depression and anxiety [1-2].

There are many different treatments plans available for acne vulgaris, including hormonal, anti-androgen, or antiseborrheic therapies as well as benzoil peroxide, retinoids, isotretinoids, keratolytic soaps, alpha hydroxy acids, azelaic acid, and salicilic acid. Acne can be treated with direct steroid injection into inflammatory cysts, microdermabrasion, chemical peels, radiofrequency, light, or laser treatments, but none of these regimens is without risks [3].

Compared to modern therapies, herbal cures typically have fewer adverse effects. Antibacterial, anti-inflammatory, and antiseptic qualities can be found in several herbs. These qualities may aid in the healing of blemishes and the decrease of germs and inflammation that cause acne. Human health, especially cosmetics, places a high value on using natural therapies, and there is a continual quest for new plant ingredients that are biologically active. Traditional medicines, such as ayurveda preparations, are sometimes categorised as supplementary and alternative therapies (CAM), have been used in acne treatment [4].

E. phaseoloides is a member of the Fabaceae family. Throughout India, the species is widely distributed, but is especially abundant in Tirupati (Andhra Pradesh), the eastern Himalayas, and East Bengal. The plant has a long history of use in Indian traditional medicine, where it is used to treat a variety of conditions including brain haemorrhage and as an emetic, anti-irritant, promoter of hair growth, and painkiller. The stem of *E. phaseoloides* is commonly used in conventional medicine due to its potent pharmacological properties[5,6]. Considering its many benefits this study aims at analysing anti acne activity of seeds of *E. phaseoloides*.

MATERIALS & METHODS

Collection of plant material

Seeds of *Entada phaseoloides* were collected in the month of October 2022, from local area of Bhopal (M.P.). Seeds of *Entada phaseoloides* were cleaned by tab water and a portion was dried at room temperature. The dried samples were ground and passed through a sieve (20 meshes) [7].

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).

Extraction procedure

58.5 gram of powdered seeds of *Entada phaseoloides* were coarsely powdered and subjected to extraction with petroleum ether by maceration method. The extraction was continued till the defatting of the material had taken place. Defatted dried powdered of *Entada phaseoloides* has been extracted with hydroalcoholic (ethanol: water: 80:20v/v) as a solvent using maceration method for 48 hrs, filtered and dried using vacuum evaporator at 40°C [8].

Determination of percentage yield

The percentage yield of each extract was calculated by dividing weight of extract by weight of powdered drug multiplied by 100.

Phytochemical analysis

Phytochemical examinations were carried out extracts as per the following standard methods [9].

Total phenol content estimation

The total phenol content of the extract was determined by the modified folin-ciocalteu method. 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/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 vortexes for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer [10].

Total flavonoids content estimation

Determination of total flavonoids content was based on aluminum chloride method. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/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% $AlCl_3$ 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.

Antimicrobial activity

Broth cultures of the pure culture isolates of those test microorganisms which are sensitive towards the phytoextracts used in present study were prepared by transferring a loop of culture into sterile nutrient

and potato dextrose broth and incubated 24 hours at 37^{o}C for bacteria and 48 hours at 24^{o}C for fungus respectively.

A loop full was taken from these broths and seeded onto sterile nutrient and potato dextrose agar plates through sterile cotton swab to develop diffused heavy lawn culture. The well diffusion method was used to determine the anti-microbial activity of the extract prepared from the *Entada phaseoloides* using standard procedure. There were 3 concentrations used which are 25, 50 and 100 mg/ml for extracted phytochemicals in studies. Its essential feature is the placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted overnight broth cultures should never be used as an inoculum. The plates were incubated 24 hours at 37^{o}C for bacteria and 48 hours at 24^{o}C for fungus respectively and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug [11].

Acute toxicity studies

Acute oral toxicity was conducted according to the method of Organization for Economic Co-operation and Development (OECD)[12]. Animals were kept fasting providing only water, hydroalcoholic extract of *Entada phaseoloides* (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[12].

Experimental designs

Group –I: Control (acne induced)

Group -II: Seeds hydroalcoholic extract of *Entada phaseoloides* (100mg/kg, p.o.)

Group –III: Seeds hydroalcoholic extract of *Entada phaseoloides* (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 140µg of heat-killed bacteria. The groups II, III and IV received 100 mg/kg and 200 mg/kg of seeds hydroalcoholic extract of *Entada phaseoloides* and Clindamycin (200 mg/kg p.o.), respectively

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 days until the 10th day.

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.

RESULTS & DISCUSSION

The yield was found to be (6.47% w/w of crude drug) of hydroalcoholic extract with solid mass of *Entada phaseoloides.* The phytochemical screening of *Entada phaseoloides* showed the presence of various classes of secondary metabolites, including alkaloids, flavonoids, saponins, tannins, terpenoids, and glycosides. The total phenolic & flavonoid content was found to be 0.536mg GAE/100mg of dry weight & 0.764mg QE/100mg of dry weight. The zone of inhibition for *P.acne* was found to be 18±0.57mm for 100mg/ml of dried extract. While for *E. coli* it was found to be 16±0.74mm. In case of *A. flavus* the zone of inhibition was found to be 8±0.86mm. Rats showed that both Clindamycin and hydroalcoholic extract of *Entada phaseoloides* were effective in reducing the severity of acne induced by Propionibacterium acnes. However, Clindamycin was found to be more effective in reducing the number of inflammatory cells in the affected skin, while *Entada phaseoloides* extract was found to be more effective in reducing the size of the lesions.

Table 1: Extractive values obtained from *Entada phaseoloides*

S. No.	Solvent	Time of extraction	% Yield
1.	Hydroalcoholic extract	48 hours	6.47%

Table 2: Preliminary phytochemical screening of *Entada phaseoloides*

S. No.	Phytoconstituents	Test Name	Hydroalcoholic extract
1	Alkaloids	Hager's Test	Absent
		Wagner Test	Absent
2	Glycosides	Legal's Test	Present
3	Carbohydrates	Fehling's Test	Present
4	Flavonoids	Lead acetate Test	Present
		Alkaline Test	Present
5	Diterpenes	Copper acetate Test	Present
6	Saponins	Froth Test	Present
7	Proteins	Xanthoproteic Test	Present
8	Phenols	Ferric Chloride Test	Present

Table 3: Estimation of total phenolic and flavonoids content of *Entada phaseoloides*

S. No	Extract	Total phenol content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1	Hydroalcoholic	0.536	0.764

Table 4: *In vitro* antimicrobial and anti acne activity of hydroalcoholic extract of *Entada phaseoloides*

S. No.	Hydroalcoholic extract	Zone of Inhibition (nm)		
		100 mg/ml	50 mg/ml	25mg/ml
1.	*Propionibacterium acnes*	18±0.57	14±0.5	11±0.74
2.	*Escherichia coli*	16±0.74	15±0.94	11±0.47
3.	*Aspergillus flavus*	8±0.86	8±0.5	7±0.57

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

Treatment	Dose	Mean thickness ±SEM				
		Day2	Day4	Day6	Day8	Day10
Control	140 µg	1.52±0.18	1.48 ± 0.15	1.32± 0.18	1.29± 0.18	1.25± 0.16
Entada phaseoloides extract	100mg/kg p.o.	1.41±0.25*	0.38±0.19*	0.25±0.18*	0.21±0.35*	0.20±0.25*
Entada phaseoloides extract	200mg/kg p.o.	1.03±0.25**	0.23±0.40**	0.18±0.35**	0.17±0.32**	0.16±0.30**
Clindamycin	200 mg/kg p.o.	0.92±0.30**	0.17±0.30***	0.09±0.30***	0.08±0.30***	0.07±0.30***

Values are expressed as the mean ± SEM of six observations. *** $P<0.001$ vs. control treatment (One-way ANOVA followed by Dunnett's test)

CONCLUSION

The study suggests that both Clindamycin and *Entada phaseoloides* extract have potential as alternative treatments for acne caused by *Propionibacterium acnes*. However, further studies are needed to confirm the efficacy and safety of these treatments in humans. It is also important to note that the use of antibiotics such as Clindamycin can contribute to the development of antibiotic resistance, and alternative treatments such as natural plant extracts may be a more sustainable option in the long term.

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