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Evaluation of Anti-Acne Activity of *Ficus carica* as an Evidence of Current Usage in Herbal Formulations

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Abstract: Ficus carica fruits & leaves have been reported to possess anti-bacterial and antifungal activity. They are also present in anti-acne formulations. However, they have not been proven for their anti-acne activity according to modern methods. The aim of the present work is to investigate whether the use of F. carica fruits & leaves in anti-acne market formulations is justified. Successive extraction of F. carica fruits and leaves was performed separately using Petroleum Ether, Chloroform, Methanol and Distilled Water. Anti-acne activity was evaluated against *Propionibacterium acnes* by measuring Zone of Inhibition of all four extracts of fruits as well as leaves using agar disc diffusion method. This was followed by calculation of Minimum Inhibitory Concentration of the most active extract using serial tube dilution method. For both leaves and fruits of F. carica, their water extracts were found to be most effective against P. acnes with a Minimum Inhibitory Concentration of 10ug/ml. The present work provides proof of anti-acne potential of F. carica fruits & leaves, which justifies their use in anti-acne formulations. Also, its activity at a much lower concentration can help in formulation development at much lower doses, and indicates that development of resistance of P. acnes against the water extract will be minimal. The water extract can be further investigated for its phytochemical composition and for isolating anti-acne phytoconstituents.

Keywords: Acne, *Ficus carica*, Minimum Inhibitory Concentration, *Propionibacterium acnes*, Zone of Inhibition.

Introduction

Ficus carica (Moraceae) is commonly known as 'Anjeer'. It possesses anti-inflammatory activity. Its leaves have rutin, ficusin, taraxasterol, stigmasterol, sitosterol, calotropenyl acetate, lupeol acetate and oleanolic acid¹. Its fruits & leaves have flavanoids which are anti-oxidant^{2, 3}. *F. carica* leaves & fruits were found to have anti-bacterial and anti-fungal activity^{3, 4}. Also, according to EWG Skin Deep® Cosmetic Database, its fruit & leaf extracts are already being used in several marketed skin formulations. However, there is no research to prove the actual potential of *F. carica* fruits and leaves against the anaerobic bacteria *Propionibacterium acnes*, which is the main acne-causing agent. The present work is aimed at evaluating anti-acne activity of *F. carica* fruits and leaves, which can provide scientific evidence to its medicinal claims.

Materials & Methods

Collection of plant material

Ficus carica leaves were collected from the medicinal garden of School of Pharmacy, RK University in August, 2014. Its dried fruits were collected in August, 2014, from local herbal store, Dhoraji, Gujarat. Herbarium was prepared and authenticated by botanist of School of Science, RK University.

Successive Extraction

F. carica leaves were dried completely in hot air oven at 50°C. 500g leaves and 500g fruits were successively extracted separately in round bottom flask using 11 each of Petroleum Ether, Chloroform, Methanol and Distilled Water at 50°C for 3h.

Anti-Microbial Screening

Agar disc diffusion method was employed. Nutrient Broth was prepared by dissolving 13g of nutrient medium in 11 distilled water and boiled to dissolve the medium completely. Fresh culture of the *Propionibacterium acnes* (Gram positive anaerobic bacteria, MTCC 1951) isolates was inoculated in Nutrient Broth and incubated for 24h at 37°C. Nutrient Agar medium was prepared by dissolving 33.9g of agar in 11 distilled water. Prepared bacterial culture (40μ I) was added in 20ml soft agar media. This media was added in the Nutrient Agar petriplate. This media was solidified and cavities were made using cup borer. The extracts (Petroleum ether, Chloroform, Methanol and Water) were added in the cavities in 50% and 100% concentration. Dimethylsulfoxide (DMSO) was used as control and Erythromycin was used as standard. The plates were then incubated at 37°C for 24h. Anti-acne activity was assayed by measuring the diameter of the Zone of Inhibition (ZOI) in mm.

Minimum Inhibitory Concentration (MIC)

It was measured by serial tube dilution technique. Extract of fruits and leaves showing the largest ZOI was taken (1mg/ml) and serial dilution of the extract (0 to 100μ g/ml) with nutrient broth for *P. acnes* inoculums was used. The test tubes were incubated for 24h at 37°C. The lowest concentration without visible growth was defined as MIC.

Results and Discussion

Zone of Inhibition



Figure 1. Zone of Inhibition of Ficus carica leaves

Cavity filled with	Pet. Ether	Chloroform	Methanol	Water Extract
	Extract (mm)	Extract (mm)	Extract (mm)	(mm)
1mg/ml extract	0.4	0.7	1.1	1.6
0.5mg/ml extract	0.5	0.7	0.4	0.5
DMSO	0.5	0.5	0.5	0.5
Erythromycin (1%)	2	2	2	2





Figure 2. Zone of Inhibition of Ficus carica fruits

 Table 2: Zone of Inhibition of Ficus carica fruits

Cavity filled with	Pet. Ether	Chloroform	Methanol	Water
	Extract (mm)	Extract (mm)	Extract (mm)	Extract (mm)
1mg/ml extract	0.3	1.1	1.5	1.6
0.5mg/ml extract	0.1	1.0	1.0	1.1
DMSO	0.5	0.5	0.5	0.5
Erythromycin	2	2	2	2
(1%)				

Minimum Inhibitory Concentration

From the observation of Zone of Inhibition of all extracts of fruits and leaves of *F. carica*, it was found that the distilled Water extract of fruits as well as leaves has the best anti-acne activity, almost comparable to that of Erythromycin in 1mg/ml concentration (Table 1, 2 and Fig. 1, 2). Hence, the Water extract was chosen to find out the Minimum Inhibitory Concentration of fruits and leaves (Table 3).

Fruits		Leaves		
Concentration of	P. acnes Growth	Concentration of	P. acnes Growth	
Water extract (µg/ml)		Water extract (µg/ml)		
0	Present	0	Present	
10	Absent	10	Absent	
20	Absent	20	Absent	
30	Absent	30	Absent	
40	Absent	40	Absent	
50	Absent	50	Absent	
100	Absent	100	Absent	

 Table 3: Minimum Inhibitory Concentration of Ficus carica

Thus, $10\mu g/ml$ is the Minimum Inhibitory Concentration of the water extract of fruits as well as leaves of *F. carica* against *P. acnes*, which is the main cause of acne. Such a low MIC indicates that much lower doses of the extract in a pharmaceutical formulation would be required to bring the desired anti-acne effect. Also, such a low MIC can help us predict that the resistance of the acne-causing *P. acnes* towards the water extract of fruits & leaves will be very less. The water extract of leaves as well as fruits contains flavanoids and polyphenols, which have been detected in *F. carica*, and which are very well-known anti-oxidant and antimicrobial agents³. Currently, the Govt. of India is laying stress on providing scientific evidence to traditional medicinal claims. This work provides evidence of anti-acne potential of *F. carica* against acne-causing *P. acnes*, which justifies its use in market formulations. The water extract can be further explored for isolating anti-acne compounds which will be beneficial to patients in acne, which is currently one of the most prevalent skin disease.

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