



# Botaniplex™ CLEAR

## Efficacy



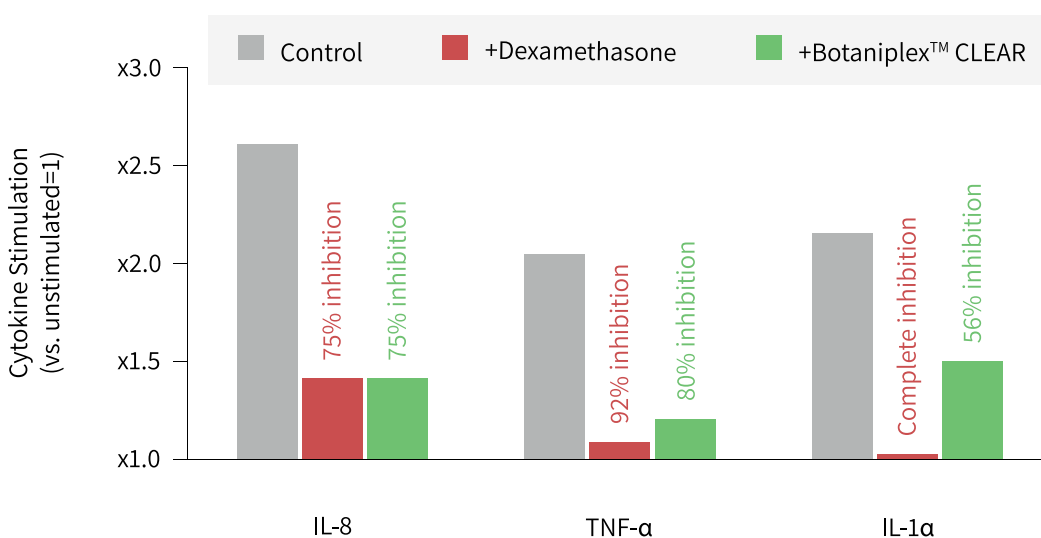
Botaniplex™ CLEAR contains numerous phytochemicals including alkaloids, anthraquinones, flavonoids, terpenoids and other phenolics. These molecules confer Botaniplex™ CLEAR with anti-inflammatory and antioxidative properties together with selective antimicrobial and prebiotic activities. These bioactivities all play a part in alleviating acne and its sequelae.

### IN VITRO EFFICACY:

#### Anti-inflammatory

Inflammation is the cause of the pain and discomfort of acne pimples, and their unsightly redness. Figure 1 shows the secretion of inflammatory cytokines from artificial skin (EpiDerm™) and natural skin. PMA or LPS induce an inflammatory response stimulating inflammatory cytokines at least 2-fold. The stimulation is curtailed by anti-inflammatory agents exemplified in this experiment by dexamethasone, a corticosteroid drug. The experiment shows that 11.5% Botaniplex™ CLEAR is also anti-inflammatory, suppressing the production of all three cytokines.

**Figure 1.** Inhibition of proinflammatory cytokines from PMA-stimulated artificial skin (IL-8 and TNF- $\alpha$ ) or natural skin (IL-1 $\alpha$ )



#### Microbiome control

Human skin is colonized by numerous microorganisms, the main commensal bacteria being *Staphylococcus epidermidis* and *Cutibacterium acnes*. These two species are mutually antagonistic, but coexist on the skin surface in a dynamic equilibrium that can shift in either direction.

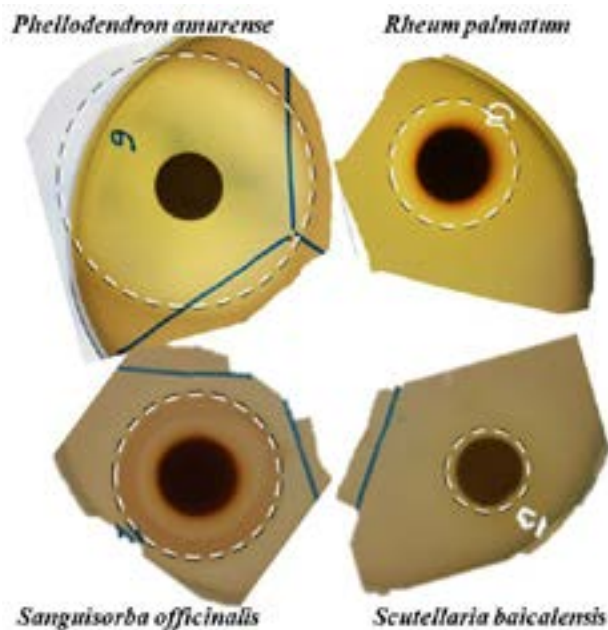


*C. acnes*, formerly classified as *Propionibacterium acnes*, is an anaerobic species; it penetrates into hair follicles to be nourished by sebum secretions but is sufficiently aerotolerant to survive also on the skin surface. *C. acnes* growth in hair follicles contributes to pore clogging and formation of acne pimples.

*S. epidermidis* is an aerobe, and thus remains mostly on the skin surface, but can in some circumstances enter the mucosa. It is rarely pathogenic. *S. epidermidis* is considered beneficial, preventing acne by curbing the excessive growth of *C. acnes* and countering inflammatory molecules that *C. acnes* produces. *S. epidermidis* also plays an important role in competing out its pathogenic cousins, *Staphylococcus capitis* and *Staphylococcus aureus*.

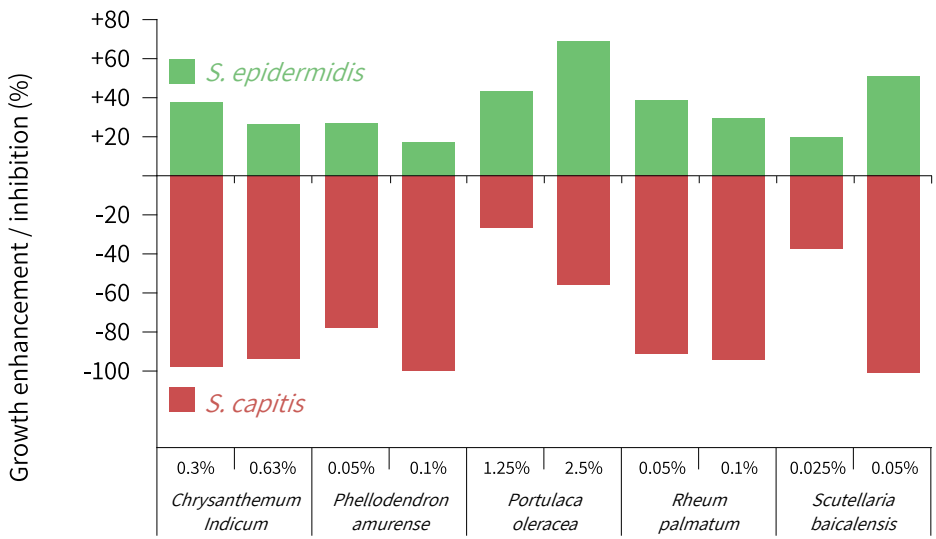
The effects of the extracts on growth of bacterial species mentioned above were tested on agar plates (“zones of inhibition”, see Figure 2) and/or by following the growth in microplates. Figure 3 emphasis the selectivity of botanicals in Botaniplex™ CLEAR, with five of them able to promote *S. epidermidis* while inhibiting *S. capitis*. Together, the results show how the botanicals help restore skin health by rebalancing the microbiome.

**Figure 2.** Agar plate disc test. Each extract (100µL) was applied to a filter disc and laid on a *C. acnes*-inoculated agar plate. White dashed rings demarcate inhibition zones.





**Figure 3.** Prebiotic and antibiotic activities of herbal extracts towards *S. epidermidis* and *S. capitis*. The bacteria were seeded in extract-laced medium in microplate wells. Growth was monitored by recording optical density ( $A_{600}$ )



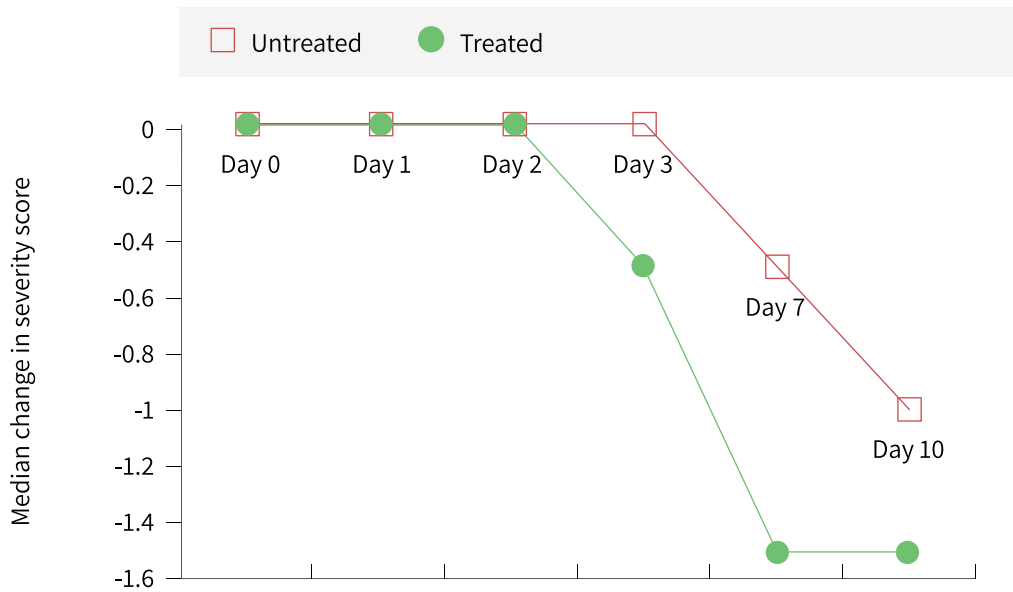
**CLINICAL EFFICACY:**

A clinical study was conducted to evaluate the tolerability and efficacy of a topical gel containing 11.5% BOTANIPLEX™ CLEAR herbal components [Barak-Shinar D, Draelos ZD. A Randomized Controlled Study of a Novel Botanical Acne Spot Treatment. *J Drugs Dermatol.* 2017 Jun 1;16(6):599-603]. The subjects (10 male, 15 female, 12 to 43 years old) were all with acne lesions distributed on both sides of the face. One side of each subject's face was subjected to application of the gel three times a day, the other side serving as a control. At baseline (day 0), lesions on each side of the face were allocated a severity score (maximum 4). The same lesions were then evaluated on days 1, 2, 3, 7 and 10.

Both treated and control lesions started with a median score of 2.5, interquartile range 2 to 3. As compared to the untreated lesions, the treated lesions displayed significantly faster reduction in severity (Figure 4), with obvious improvement in some subjects visible already after just 5 applications of the gel (Figure 5).



**Figure 4.** Acne severity scores of subjects treated topically with gel containing Botaniplex™ CLEAR. Opposite sides of the face in each patient served as treatment and control (details in text).



**Figure 5.** Photographs of lesions on study subjects before and after application of a gel containing BOTANIPLEX™ CLEAR herbal components.



In summary, Botaniplex™ CLEAR has anti-inflammatory and microbiome-balancing bioactivities, as demonstrated *in vitro* cellular and bacteriological experiments. These bioactivities are presumably the source of the proven clinical efficacy of Botaniplex™ CLEAR against acne.