

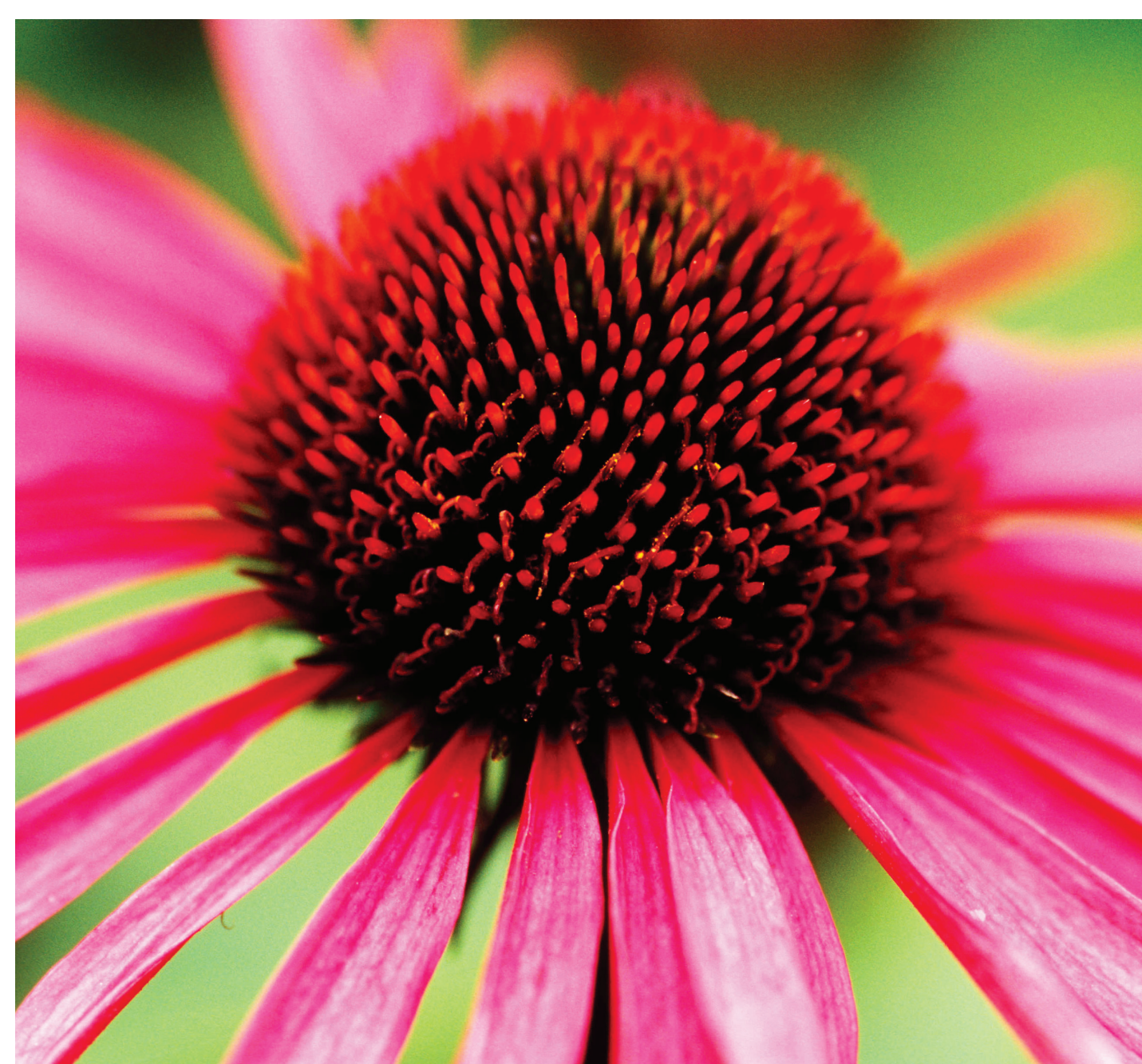
## TARGETING GENES RELATED TO SKIN FIRMNESS- AN IN-VITRO APPROACH

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### INTRODUCTION

Gene expression in the skin is regulated by thousands of genes. Genes related to skin aging may fall into the following categories: cellular proliferation, protection, structure, hydration and pigmentation! Firm skin is physically taut having few visible wrinkles and high elasticity. The loss of skin structure or elasticity is usually one of the first and most noticeable sign of skin aging. Research has shown that Commiphora Mukul Resin Extract, Echinacea Angustifolia Extract, Centella Asiatica Extract and Octyldodecyl Olivatate Extract have significant anti-aging skin benefits.<sup>2,3,4</sup> A recent combination of Echinacea Angustifolia Extract, Centella Asiatica Extract and Octyldodecyl Olivatate was shown to have clinical benefits on skin firmness. Clinical studies of Commiphora Mukul Resin Extract from India's Mukul Myrrh tree also show benefits on skin firmness when applied topically. In this investigation, genes were selected based on published literature for their relevance in influencing skin firmness. The effects of a blend of Commiphora Mukul Resin Extract, Echinacea Angustifolia Extract, Centella Asiatica Extract and Octyldodecyl Olivatate were measured for their effects on these genes to further substantiate the potential benefits of these materials on skin firmness.



The Echinacea plant



Centella Asiatica plant

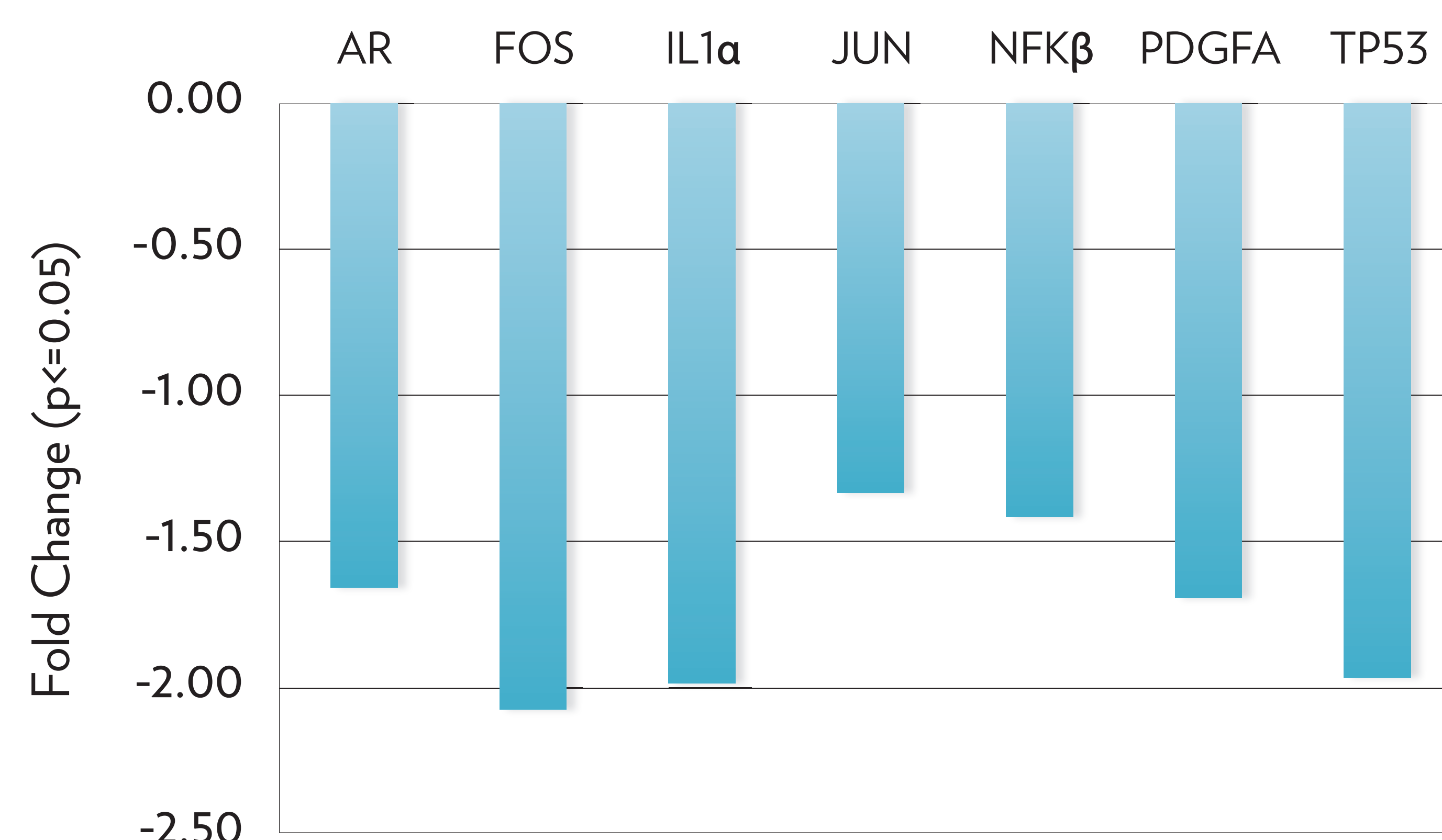


Figure 1. qPCR data illustrating a decrease in expression of genes associated with skin structure deterioration after 24 hours of incubation with Commiphora Mukul Resin Extract (see Table 1 for full description of genes and functions).

### MATERIALS AND METHODS

Epidermal full-thickness skin cultures were obtained from MatTek (Ashland, MA, USA). These cultures are comprised of normal human-derived epidermal keratinocytes and normal human-derived dermal fibroblasts. A combination of Echinacea Angustifolia Extract, Centella Asiatica Extract and Octyldodecyl Olivatate (1%), and Commiphora Mukul Resin Extract (1%) were separately applied to the cultures for 24 hours. Cultures incubated without the extracts were used as control. RNA was extracted from the cultures and converted to cDNA using the High Capacity Transcription Kit from Life Technologies (Foster City, CA USA). Reactions were performed according to manufacturer instructions. Custom Taqman Low Density Arrays (TLDA) were created using Life Technologies validated gene expression assays. Each TLDA card contained 379 target genes and five common endogenous control genes. An Applied Biosystems 7900HT (Applied Biosystems, Foster City, CA USA) was used for amplification and fluorescence detection. Data analysis for qPCR was carried out according to the RQ analysis method using RQ Manager and STATMINER (v3.1) software programs.

### RESULTS

Gene	Gene Symbol	Role in Skin Structure
AR	Androgen Receptor	Collagen Breakdown <sup>5</sup>
FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog	Promotes MMP's <sup>6</sup>
IL1α	Interleukin 1 Alpha	Induced Collagenase <sup>7</sup>
JUN	JUN	Promotes MMP's <sup>8</sup>
NFKβ	Nuclear Factor Kappa Beta	Diverse effects <sup>9</sup>
PDGFA	Platelet-derived growth factor subunit A	UV induced collagenase <sup>10</sup>
TP53	Tumor protein p53	Suppressor of collagen gene <sup>11</sup>
FOXO3	Forkhead Box O <sup>3</sup>	Helps prevent degradation <sup>12</sup>
LOX	Lysyl Oxidase	Strengthens ECM <sup>13</sup>
KLK14	Kallikrein 14	Promotes cellular turnover <sup>14</sup>
PKP2	Plakophilin 2	Strengthens ECM <sup>15</sup>

Table 1. Summary of key skin structure genes investigated in this study

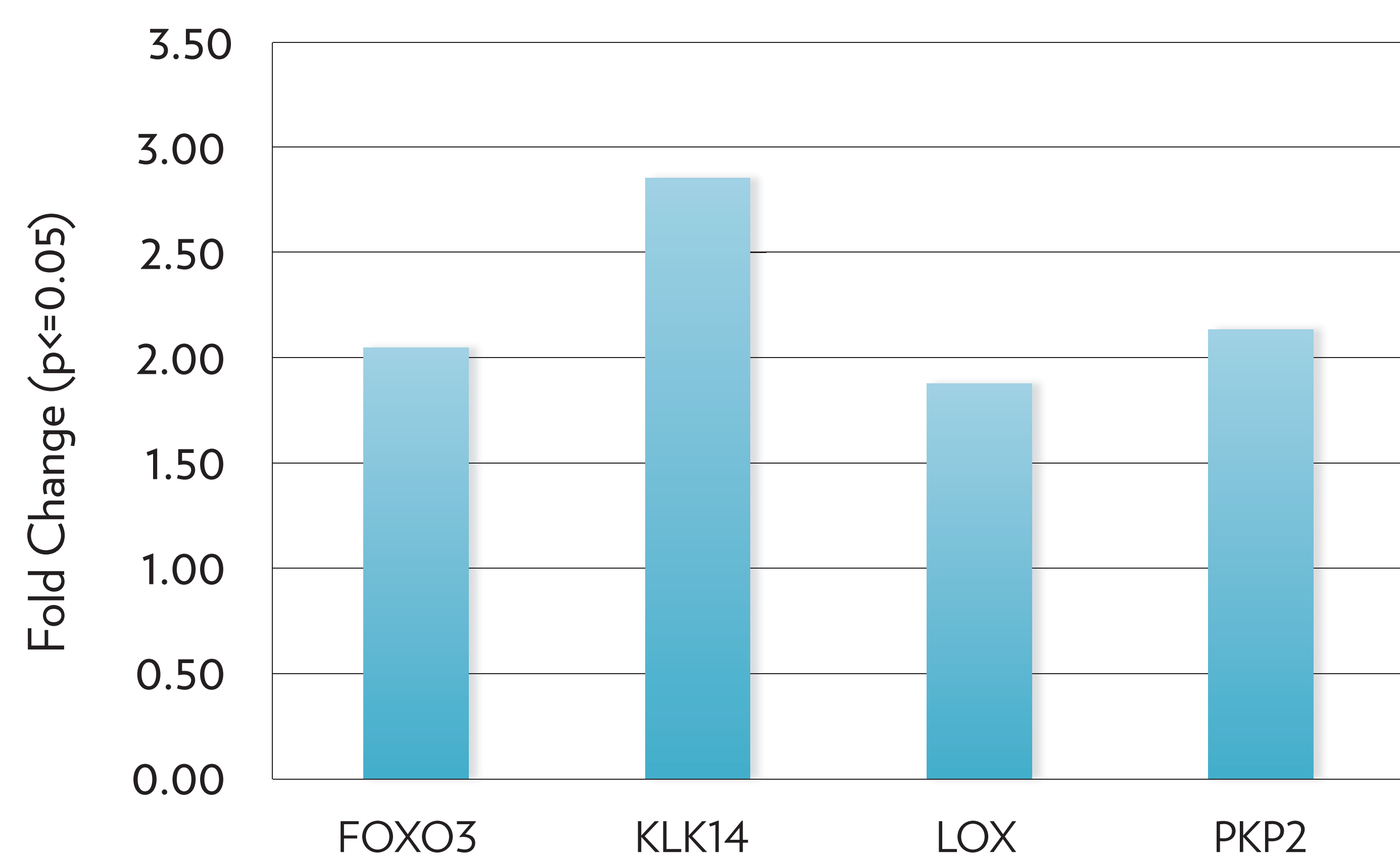


Figure 2. qPCR data illustrating an increase in expression of genes associated with skin structure integrity after 24 hours of incubation with a combination Echinacea Angustifolia Extract, Centella Asiatica Extract and Octyldodecyl Olivatate (see Table 1 for full description of genes and functions).

### DISCUSSION

Results showed that a combination of Commiphora Mukul Resin Extract, Echinacea Angustifolia Extract, Centella Asiatica Extract and Octyldodecyl Olivatate regulated the expression of key genes associated with the maintenance of skin structure proteins. Commiphora Mukul Resin Extract downregulated key genes related to skin structure degradation. These include JUN, FOS, IL1A, AR, PDGFA and TP53. One key regulator that was also downregulated is NFKB. NFKB is a key gene that is activated during oxidative stress which is responsible for the degradation of key skin structure proteins relevant for the maintenance of firm looking skin.<sup>9</sup> By downregulating this gene, Commiphora Mukul Resin Extract may be acting as an inhibitor of NFKB. A combination of Echinacea Angustifolia Extract, Centella Asiatica Extract and Octyldodecyl Olivatate increased the regulation of the gene FOXO3, KLK14, LOX and PKP2. These genes have been researched to positively impact the integrity of the extra-cellular matrix, resulting in firmer and more robust skin appearance. It is proposed that a combination of Commiphora Mukul Resin Extract, Echinacea Angustifolia Extract, Centella Asiatica Extract and Octyldodecyl Olivatate in a topical cosmetic formulation may be able to increase skin firmness clinically by decreasing key genes responsible for the degradation of skin structure proteins and increasing the expression of genes associated with skin integrity. Recent unpublished clinical studies involving the combination of all three of these materials in a finished topical formulation seemed to support the hypothesis that skin firmness is improved when these extracts are combined in this manner.

### CONCLUSION

The findings from this study suggest a possible role of a combination of Commiphora Mukul Resin Extract, Echinacea Angustifolia Extract, Centella Asiatica Extract and Octyldodecyl Olivatate on enhancing skin firmness when applied topically.

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