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# USE OF THE MatTek EPI-100 IN VITRO SYSTEM TO SCREEN ANTIOXIDANT EFFICACY

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## Abstract

An in vitro method to evaluate the efficacy of antioxidants and antioxidant formulations is presented. The premise of this research is that exposure of human skin equivalents, specifically the commercially available MatTek EPI-100 skin model, to ultraviolet B (UVB) irradiation stimulates the production of oxygen radicals in tissue, which, over time, will lead to an increased production of prostaglandins ($PGE_2$). The literature records that an increase in oxygen radical production leads to an increase in prostaglandin production.[1-7] The method presented measures two endpoints: conversion of (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenoltetrazolium) bromide (MTT) and $PGE_2$ production, the marker for antioxidancy. Based on these results, it is possible to rank order test agents and determine relative efficacy. This method has proved to be a reproducible and accurate method to assess differences in efficacy among potential antioxidants.

## Introduction

Antioxidants have become a point of focus for the cosmetic and personal care industries. Currently, few nonanimal antioxidant evaluation methods are available. Presently available nonanimal models are not representative of skin structure. Available methods include monolayer cell culture and chemical analysis

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of essential oil oxidation.[8,9] While monolayer cell culture models are fair indicators of antioxidant activity, they do not take into account the presence of a stratum corneum. Antioxidants may show activity in monolayer and not on three-dimensional constructs due to the fact that some materials may not be able to penetrate the stratum corneum. In addition, monolayer cell culture requires diluting the antioxidant into culture medium, which may alter the product's efficacy. The presented method allows dosing of neat test material. This protocol was developed to provide an animal alternative test method with a rapid, easy, reliable, and cost-effective means of screening formulations and raw materials during the development process to assess their efficacy against known standards or competitive products.

## Materials and Methods

### *Test Materials*

Thirteen materials were evaluated. These materials were mixed tocopherols, water-soluble licorice extract, quercetin, potassium glycyerrhinate, delta tocopherol, actiphyte of white grape, magnesium ascorbyl phosphate, green tea BG extract, tomato extract, actiphyte of grapefruit, liposomal superoxide dismutase (SOD), experimental formula A, and green tea AMT extract. All of the products were supplied by the Shaklee Corporation (Hayward, CA). These materials were included to evaluate known antioxidants (liposomal SOD, delta tocopherol, and mixed tocopherol) as well as antioxidants of interest to the industry.

### *Tissue System and Assay Kits*

The tissue system selected for this work was the MatTek Corporation's EpiDerm (EPI-100). This economical and repeatable system consists of normal human-derived epidermal keratinocytes that have been cultured to form a multilayered, highly differentiated model of the human epidermis. Keratinocytes are cultured on specially prepared permeable cell culture inserts that allow attainment of levels of differentiation on the cutting edge of in vitro skin technology. In ultrastructural terms the EPI-100 closely parallels human skin, thus providing a useful in vitro substrate to assess dermal toxicity. This method is also applicable to the Advanced Tissue Sciences' model ZK1301 system with appropriate protocol modifications.

The $PGE_2$ assay was performed using a kit from Perseptive Diagnostics. The media used to support cell viability during testing (spent media) were collected after testing and diluted in enzyme immunoassay (EIA) phosphate buffer prior to analysis in this kit. This was done to dilute the levels of $PGE_2$ within the range of the kit standard curve. The results from the analysis were then corrected for dilution.

### *Solar Simulator*

A single-port solar simulator from the Solar Light Company was utilized in this study. The UVB detector was used and the dosage of UVB measured in minimum erythemogenic dose (MED)/h/cm$^2$. This is a standard unit of measure for this solar simulator[10] and is equivalent to 21 mJ/cm$^2$.

### *Cell Viability Measurements*

Cell viability was measured using the MTT assay. The MTT assay is a colorimetric analysis of cell metabolic activity. Reduction of MTT by mitochondria results in the formation of insoluble blue formazan crystals that are extracted from the cells with isopropanol and quantitated spectrophotometrically. The intensity of the blue color is directly proportional to the metabolic activity of the cells and inversely proportional to the toxicity of the test material.[11-13]

### *MTT Compatibility*

A compatibility test was carried out prior to dosing the test material(s) in the test system. An aliquot of each test material was mixed with an equal volume of 1 mg/ml MTT solution, in a birosilicate glass test tube. Tubes were capped and incubated in the dark at room temperature for approximately 3 h unless conversion occurred sooner. Evidence of a color change indicated that the test material may spontaneously reduce MTT, resulting in a false reaction. If the test material was not MTT-compatible, blank tissue holders were dosed (along with the test system), rinsed, and residual MTT conversion determined vs. tissue holders not treated with test material and exposed to MTT. Background readings were subtracted from the respective test materials if any were observed. Test agents delta tocopherol and mixed tocopherol were found to be MTT-incompatible. The MTT conversion values of these agents were subtracted from the conversion values of the tissue.

### *In Vitro Method*

This method is suitable for antioxidants or antioxidant-containing formulations. The $PGE_2$ endpoint is most reliable when noncytotoxic test agents are used.

### *Cytotoxicity Evaluation*

Prior to testing in the antioxidant method, test agents were screened for cytotoxicity and their ability to stimulate $PGE_2$ release by themselves. Test agents that stimulate $PGE_2$ production may show elevated levels of $PGE_2$ in the antioxidant assay, which is not attributable to a lack of antioxidant efficacy.

Tissues were transferred from the shipping containers to individual wells of six-well plates containing 0.9 ml assay medium per well. This assay medium was supplied by the MatTek Corporation and was serum free. The tissues were preincubated for 1 h at 37°C and 5% $CO_2$ to equilibrate the system after shipping. This preincubation also allowed the removal of any $PGE_2$ accumulated in the tissue during shipping. After this preincubation, the assay medium was aspirated and replaced with 0.9 ml fresh 37°C assay medium. All testing was done in triplicate. The negative control was tissue dosed with 100 $\mu$l deionized water. The positive control was tissue dosed with 100 $\mu$l 1% Triton X-100. The experimental tissues used to judge cytotoxicity were dosed with 100 $\mu$l test agent. Once dosed, tissue was placed into a 37°C and 5% $CO_2$ incubator for 24 h. Individual tissues were dosed at 30 s intervals. At the end of 24 h exposure, tissue was removed from the incubator and rinsed free of test agent. Tissue viability was measured by placing the tissue into 300 $\mu$l 1 mg/ml MTT solution prepared in assay medium for 3 h at 37°C and 5% $CO_2$. The spent medium was collected for analysis of $PGE_2$. After 3 h incubation with MTT, tissue was rinsed and extracted in 2 ml isopropanol for at least 2 h. Once extracted, 200 $\mu$l extract from each well was transferred to individual wells of a 96-well plate and absorbance determined spectrophotometrically at 570 nm against a isopropanol blank. Tissue viability was determined by dividing the average optical density from experimental or positive control tissues by the average optical density of negative control tissues. This number was then multiplied by 100 to arrive at the percentage viability of experimental tissue compared to negative controls.

### *Antioxidant Method*

Tissues were transferred from the shipping containers to individual wells of six-well plates containing 0.9 ml assay medium per well. This assay medium was supplied by the MatTek Corporation and was serum free. The tissues were preincubated for 1 h at 37°C and 5% $CO_2$ to equilibrate the system after shipping. This preincubation also allowed the removal of any $PGE_2$ accumulated in the tissue during shipping. After this preincubation, the assay medium was aspirated and replaced with 0.9 ml fresh 37°C assay medium. All testing was done in triplicate. The chosen dosages of UVB irradiation in this assay were 1.5 and 3.0 MED/hr/cm$^2$. The negative control tissues were those not dosed with test agent and not irradiated with UVB. The positive control tissues were those not treated with test agent but with UVB irradiation. Experimental tissues were those irradiated with UVB and then dosed with test agent. Dosing proceeded by exposing tissue to UVB irradiation using a solar simulator. Tissue was mounted onto a 24-well plate filled with 2% agarose and irradiated. The agarose helped to prevent the tissue drying out during irradiation. In general, irradiation took approximately 1–3 min (depending on dose) per tissue piece. Immediately after irradiation, the tissue was placed back into its respective well from the six-well plate and

dosed with 100 μl test agent. Once dosed, the tissue was placed into a 37°C and 5% $CO_2$ incubator for 24 h. Tissues were timed individually. At the end of 24 h exposure, tissue was removed from the incubator and rinsed free of test agent. Tissue viability was measured by placing the tissue into 300 μl 1 mg/ml MTT solution prepared in assay medium for 3 h at 37°C and 5% $CO_2$. The spent medium was collected for analysis of $PGE_2$. After 3 h incubation with MTT, tissue was rinsed and extracted in 2 ml isopropanol for at least 2 h. Once extracted, 200 μl extract from each well was transferred to individual wells of a 96-well plate and absorbance determined spectrophotometrically at 570 nm against a isopropanol blank. Tissue viability was determined by dividing the average optical density from experimental or positive control tissues by the average optical density of negative control tissues. This number was then multiplied by 100 to arrive at the percentage viability of experimental tissue compared to negative controls.

## Results

The endpoint for cytotoxicity in this assay was conversion of MTT. The levels of $PGE_2$ produced in response to dosed test agents were also evaluated. Prostaglandins are mediators of inflammation and are derived from arachidonic acids taken from phospholipids in the cell membrane. These mediators are responsible for anaphylactic effects such as vasodilation, increased vascular permeability, and hyperalgesia. The concentration of prostaglandins in tissue correlates with inflammation-related effects such as erythema and edema.

### *Cytotoxicity Screen*

Mixed tocopherols and delta tocopherols were MTT incompatible and were dosed onto blank tissue holders. The resulting conversion as a function of the test materials alone was high enough to reduce cell viability readings into the negative range when subtracted from the tissue's MTT conversion values. A visual inspection of the tissue showed that the tissue had taken up and converted the MTT indicating strong cell viability. All other test agents caused no significant cytotoxicity in the cytotoxicity screen. The results for the cytotoxicity assays are shown in Figure 1.

$PGE_2$ analysis showed low levels of prostaglandin production, in most cases, in response to materials that were not cytotoxic. In the case of liposomal SOD, green tea BG extract, actiphyte of grapefruit, magnesium ascorbyl phosphate, and experimental formula A, however, elevated levels of $PGE_2$ were noted but no cytotoxicity was seen. This is not unexpected since $PGE_2$ release is an indicator of inflammation and cell stress but not necessarily cytotoxicity. A test material

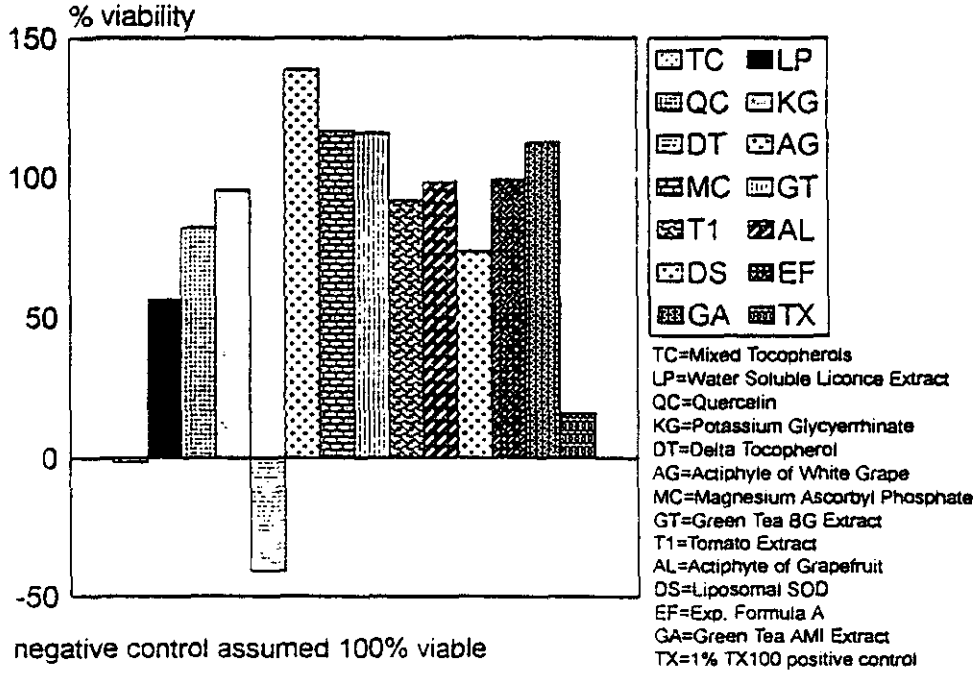


*Figure 1.* Cytotoxicity screen: MTT conversion.

may elicit an increase in $PGE_2$ production but may not be cytotoxic. The levels of $PGE_2$ produced in tissue as a result of test material exposure are shown in Figure 2.

### *Antioxidant Assay*

Results indicate test materials possess a range of antioxidant activity. The results of the MTT assay indicate that many of these materials are able to enhance the recovery of tissue after an insult with UVB irradiation. This is supported with statistical evidence showing differences between experimental tissue and positive control tissue in some cases.

Viability measurements were done on tissue after exposure in the antioxidant assay using the MTT assay. Although MTT is not an indicator of antioxidant efficacy, it may be a marker useful in showing beneficial effects of test material after UVB damage. If tissue treated with test material prior to UVB irradiation shows greater viability than tissue treated with UVB irradiation only, the test material may be implicated in reducing the effects of UVB injury. These MTT conversion results are presented in Figure 3.

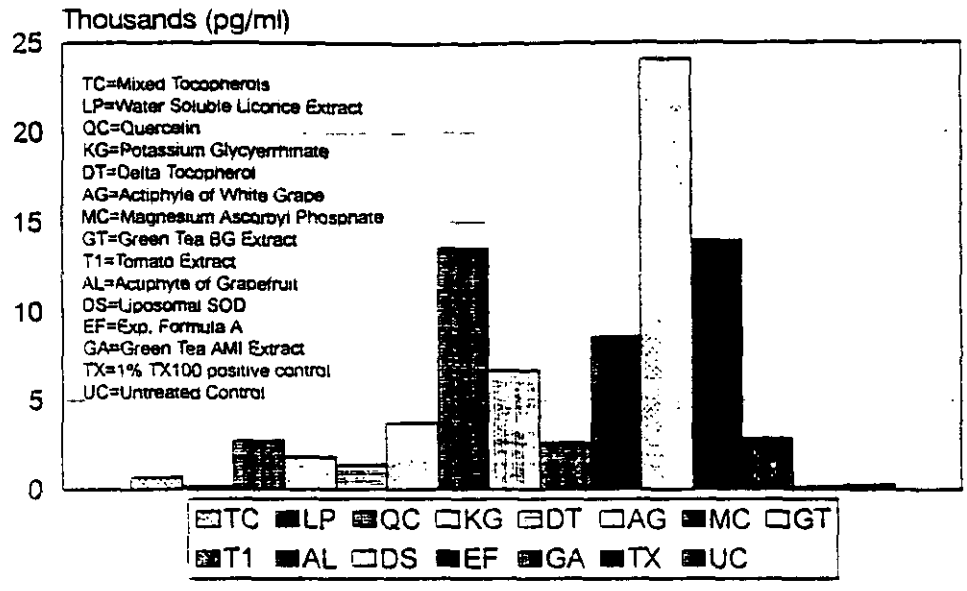


*Figure 2.* Cytotoxicity screen: PGE production as a result of test material.

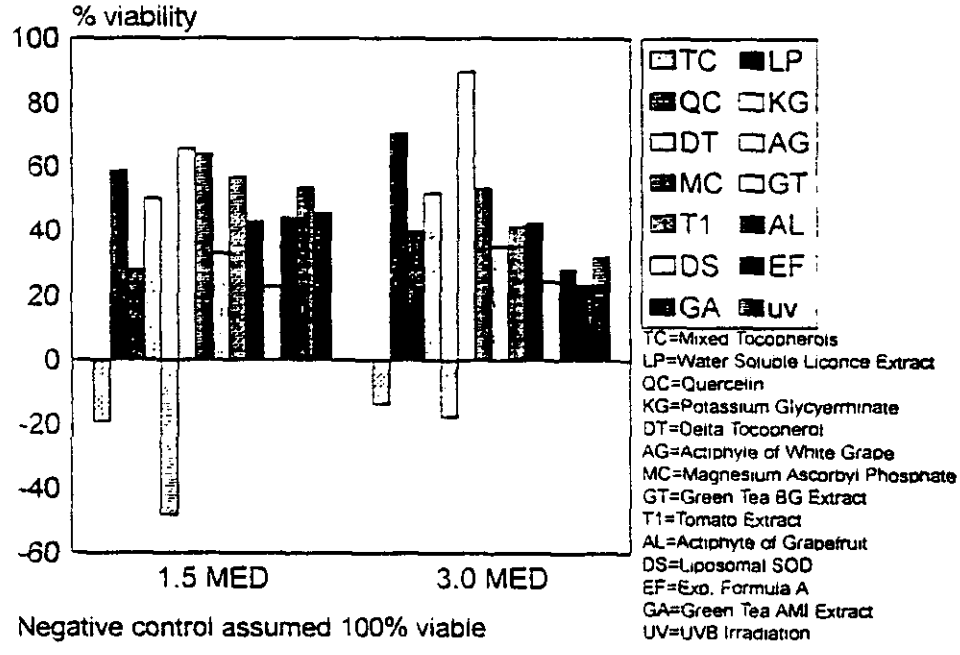


*Figure 3.* Antioxidant method: Cytotoxicity of test material after UVB irradiation.

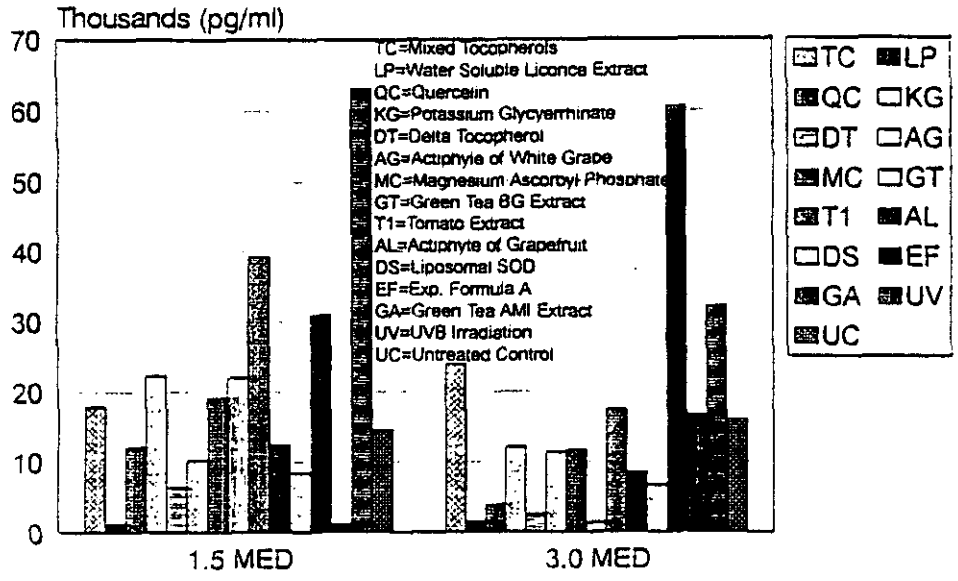


*Figure 4.* Antioxidant method: PGE production after irradiating with UVB and dosing with test material.

The $PGE_2$ assay, the endpoint for antioxidant activity, shows varying degrees of test material efficacy. At 1.5 MED/h/cm$^2$, all test agents were able to maintain $PGE_2$ production levels lower than those seen with the positive control. At 3.0 MED/h/cm$^2$, however, the test agent experimental formula A was unable to reduce the levels of $PGE_2$ production below the positive control. The results of the positive control tissue indicate that a dosage of 1.5 MED/h/cm$^2$ is optimum in providing the most $PGE_2$ release in this tissue. The $PGE_2$ results for the antioxidant assay are presented in Figure 4.

## Conclusion

### *Cytotoxicity Screen*

Because none of the test agents were cytotoxic or produced levels of $PGE_2$ notably greater than the background levels of negative control tissue, the results of the antioxidant assay can be attributed to test product efficacy.

### *Antioxidant Assay*

The controls included in this method responded as expected. The literature records the fact that delta tocopherol is more efficacious than mixed tocopherol.

This was noted in the results of this method. Furthermore, superoxide dismutase is known to be an effective antioxidant. This was also noted using this method. The experimental formulations all showed varying degrees of efficacy.

## Discussion

While differences in test agents may be noted with this method, it is important to consider the effects of the same products in vivo. This method is best utilized as a screen for potential formulas or new antioxidants. It is important to consider the correlation between different endpoints for a specific material. Often, a material is capable of reducing $PGE_2$ production in response to test agent and will also be able to enhance tissue viability compared to positive controls in the MTT assay.

This method is limited by the requirement that test materials be noncytotoxic after 24 hr incubation. This is required since differences in $PGE_2$ production in this assay are most easily seen after about 24 hours incubation.

It should be noted that the materials in this method were dosed after UVB irradiation. This is important since many antioxidant containing formulations also contain sunscreens that would prevent the penetration of UVB into the tissue. By dosing test material after irradiation, the problem of phototoxicity, as seen with some materials, is avoided.

In many cases, cytotoxic compounds are also low or high pH materials. Cytotoxicity with these materials may be reduced or eliminated by adjusting the pH to near neutral. This adjustment often has no impact on antioxidant efficacy and will allow materials such as alpha hydroxy acid compounds to be easily screened for antioxidant efficacy. Additional research is being conducted to overcome this limitation. Methods are being developed to deal with products that show cytotoxicity.

## Statistical Analysis

Statistical analysis was done on the MTT and $PGE_2$ results from the antioxidant assay. These results are reported in Tables 1–4.

The statistical analysis of MTT results, at both 1.5 and 3.0 MED/h/cm$^2$, was done to determine if increases in tissue viability in response to being dosed with test material after a dose with UVB were statistically different from tissue dosed with UVB only. These MTT results are presented in Tables 1 and 2.

Analysis of $PGE_2$ data, at both 1.5 and 3.0 MED/h/cm$^2$, was done to determine if differences between test materials were statistically significant. The $PGE_2$ results are presented in Tables 3 and 4.

*Table 1.* Statistical Analysis of 1.5 MED-MTT Conversion Data

| | TC | LP | QC | KG | DT | AG | MC | GT | TI | AL | DS | EF | GA | UV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TC | N/A | 0.05 | 0.05 | 0.05 | – | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| LP | 0.05 | N/A | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| QC | 0.05 | 0.05 | N/A | – | 0.05 | 0.05 | – | – | – | – | – | – | 0.1 | – |
| KG | 0.05 | 0.1 | 0.05 | N/A | 0.05 | 0.05 | – | 0.1 | 0.1 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| DT | 0.05 | 0.05 | 0.05 | 0.05 | N/A | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.1 | 0.05 | 0.1 | 0.05 |
| AG | 0.05 | – | 0.05 | 0.05 | 0.05 | N/A | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| MC | 0.05 | – | 0.05 | – | 0.05 | 0.05 | N/A | 0.1 | – | 0.1 | 0.05 | 0.05 | 0.05 | 0.05 |
| GT | 0.05 | 0.05 | – | 0.1 | 0.05 | 0.05 | 0.1 | N/A | – | – | – | – | – | – |
| TI | 0.05 | – | 0.05 | 0.1 | 0.05 | 0.05 | – | – | N/A | – | 0.05 | – | 0.05 | – |
| AL | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.1 | – | – | N/A | 0.05 | 0.05 | 0.05 | 0.05 |
| DS | 0.05 | 0.05 | – | 0.05 | 0.1 | 0.05 | 0.05 | – | 0.05 | 0.05 | N/A | – | – | – |
| EF | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | – | – | 0.05 | – | N/A | – | – |
| GA | 0.05 | 0.1 | 0.05 | 0.05 | 0.1 | 0.05 | 0.05 | – | 0.05 | 0.05 | – | – | N/A | – |
| UV | 0.05 | – | – | 0.05 | 0.05 | 0.05 | 0.05 | – | – | 0.05 | – | – | – | N/A |

Results of t-test to evaluate statistical differences between samples.

TC, mixed tocopherol; LP, water soluble licorice extract; QC, quercetin; KG, potassium glycyerrhinate; DT, delta tocopherol; AG, actiphyte of white grape; MC, magnesium ascorbyl phosphate; GT, green tea BG extract; TI, tomato extract; AL, actiphyte of grapefruit; DS, liposomal SOD; EF, experimental formula A; GA, green tea AMI extract; UV, positive control (tissue irradiated with 1.5 MED and undosed with test material).

–, no statistical significance; 0.05, statistical significance at the 95% confidence level; 0.1, statistical significance at the 90% confidence level.

The number of replicates for statistical analysis was 3. This is a small sample size for statistical analysis.

The number of replicates for statistical analysis was 3. This is a small sample size for statistical analysis.





*Table 2.* Statistical Analysis of 3.0 MED-MTT Conversion Data

| | TC | LP | QC | KG | DT | AG | MC | GT | TI | AL | DS | EF | GA | UV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TC | N/A | 0.05 | 0.05 | 0.05 | – | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| LP | 0.05 | N/A | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| QC | 0.05 | 0.05 | N/A | – | 0.05 | 0.05 | – | – | – | – | – | – | 0.1 | – |
| KG | 0.05 | 0.05 | – | N/A | 0.05 | 0.05 | – | 0.1 | 0.1 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| DT | – | 0.05 | 0.05 | 0.05 | N/A | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.1 | 0.05 | 0.1 | 0.05 |
| AG | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | N/A | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| MC | 0.05 | 0.05 | – | – | 0.05 | 0.05 | N/A | 0.1 | – | 0.1 | 0.05 | 0.05 | 0.05 | 0.05 |
| GT | 0.05 | 0.05 | – | 0.1 | 0.05 | 0.05 | 0.1 | N/A | – | – | – | – | – | – |
| TI | 0.05 | 0.05 | – | 0.1 | 0.05 | 0.05 | – | – | N/A | – | 0.05 | – | 0.05 | – |
| AL | 0.05 | 0.05 | – | 0.05 | 0.05 | 0.05 | 0.1 | – | – | N/A | 0.05 | 0.05 | 0.05 | 0.05 |
| DS | 0.05 | 0.05 | – | 0.05 | 0.1 | 0.05 | 0.05 | – | 0.05 | 0.05 | N/A | – | – | – |
| EF | 0.05 | 0.05 | – | 0.05 | 0.05 | 0.05 | 0.05 | – | – | 0.05 | – | N/A | – | – |
| GA | 0.05 | 0.05 | 0.1 | 0.05 | 0.1 | 0.05 | 0.05 | – | 0.05 | 0.05 | – | – | N/A | – |
| UV | 0.05 | 0.05 | – | 0.05 | 0.05 | 0.05 | 0.05 | – | – | 0.05 | – | – | – | N/A |

See legend for Table 1.

*Table 3.* Statistical Analysis of 1.5 MED-$PGE_2$ Production Data

| | TC | LP | QC | KG | DT | AG | MC | GT | TI | AL | DS | EF | GA | UC | UV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TC | N/A | 0.05 | – | – | 0.1 | – | – | – | – | – | – | 0.1 | 0.05 | – | 0.05 |
| LP | 0.05 | N/A | 0.05 | 0.05 | 0.1 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | – | 0.05 | 0.05 |
| QC | – | 0.05 | N/A | – | – | – | – | – | 0.1 | – | – | 0.05 | 0.1 | – | 0.05 |
| KG | – | 0.05 | – | N/A | 0.05 | 0.1 | – | – | – | – | 0.1 | – | 0.05 | – | 0.05 |
| DT | 0.1 | 0.1 | – | 0.05 | N/A | – | 0.05 | – | 0.05 | – | – | 0.05 | – | 0.1 | 0.05 |
| AG | – | 0.05 | – | 0.1 | – | N/A | 0.1 | – | 0.1 | – | – | 0.05 | 0.1 | – | 0.05 |
| MC | – | 0.05 | – | – | 0.05 | 0.1 | N/A | – | – | – | 0.05 | 0.1 | 0.05 | – | 0.05 |
| GT | – | 0.05 | – | – | – | – | – | N/A | – | – | – | – | – | – | 0.05 |
| TI | – | 0.05 | 0.1 | – | 0.05 | 0.1 | – | – | N/A | 0.1 | 0.05 | – | 0.1 | 0.1 | – |
| AL | – | 0.05 | – | – | – | – | – | – | 0.1 | N/A | – | 0.05 | 0.1 | – | 0.05 |
| DS | – | 0.05 | – | 0.1 | – | – | 0.05 | – | 0.05 | – | N/A | 0.05 | 0.05 | – | 0.05 |
| EF | 0.1 | 0.05 | 0.05 | – | 0.05 | 0.05 | 0.1 | – | – | 0.05 | 0.05 | N/A | 0.05 | 0.05 | 0.05 |
| GA | 0.05 | – | 0.1 | 0.05 | – | 0.1 | 0.05 | – | 0.1 | 0.1 | 0.05 | 0.05 | N/A | 0.05 | 0.05 |
| UC | – | 0.05 | – | – | 0.1 | – | – | – | 0.1 | – | – | 0.05 | 0.05 | N/A | 0.05 |
| UV | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | – | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | N/A |

See legend for Table 1.

*Table 4.* Statistical Analysis of 3.0 MED-$PGE_2$ Production Data

| | TC | LP | QC | KG | DT | AG | MC | GT | TI | AL | DS | EF | GA | UC | UV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TC | N/A | 0.1 | 0.1 | – | – | – | – | 0.1 | – | – | – | – | – | 0.1 | – |
| LP | 0.1 | N/A | 0.05 | 0.1 | – | 0.05 | 0.05 | – | 0.05 | 0.1 | 0.1 | 0.05 | 0.05 | – | 0.05 |
| QC | 0.1 | 0.05 | N/A | – | – | 0.1 | 0.05 | – | 0.05 | – | – | 0.05 | 0.05 | – | 0.05 |
| KG | – | 0.1 | – | N/A | – | – | 0.1 | 0.1 | – | – | – | 0.05 | – | – | – |
| DT | – | – | – | – | N/A | – | 0.05 | – | – | – | – | 0.05 | – | – | 0.1 |
| AG | – | 0.05 | 0.1 | – | – | N/A | 0.1 | 0.05 | – | – | – | 0.05 | – | – | 0.1 |
| MC | – | 0.05 | 0.05 | 0.1 | 0.05 | 0.1 | N/A | 0.05 | 0.1 | 0.05 | 0.05 | – | 0.1 | – | – |
| GT | 0.1 | – | – | 0.1 | – | 0.05 | 0.05 | N/A | 0.05 | – | – | 0.05 | 0.05 | – | 0.05 |
| TI | – | 0.05 | 0.05 | – | – | – | 0.1 | 0.05 | N/A | – | 0.1 | 0.05 | – | – | – |
| AL | – | 0.1 | – | – | – | – | 0.05 | – | – | N/A | – | 0.05 | – | – | 0.1 |
| DS | – | 0.1 | – | – | – | – | 0.05 | – | 0.1 | – | N/A | 0.05 | – | – | 0.1 |
| EF | – | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | – | 0.05 | 0.05 | 0.05 | 0.05 | N/A | 0.05 | – | – |
| GA | – | 0.05 | 0.05 | – | – | – | 0.1 | 0.05 | – | – | – | 0.05 | N/A | – | – |
| UC | 0.1 | – | – | – | – | – | – | – | – | – | – | – | – | N/A | – |
| UV | – | 0.05 | 0.05 | – | 0.1 | 0.1 | – | 0.05 | – | 0.1 | 0.1 | – | – | – | N/A |

See legend for Table 1.

Differences were analyzed using the student's t-test. Products are listed on both the horizontal and vertical axis of Tables 1–4. Each test material on the vertical axis was compared to the remaining test materials listed on the horizontal axis and any statistically significant differences noted as described in the legend of these tables.

## References


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