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Research Article

Effect of Quercetin and Zinc Oxide Concentrations on pH and *In Vitro* Activity of Sunscreen Lipstick

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Abstract

Quercetin, a naturally occurring flavonoid, exhibits significant antioxidant properties, making it a promising candidate for photoprotective applications. Concurrently, zinc oxide is a well-established physical blocker of ultraviolet A (UV-A) radiation. This study aimed to optimize the synergistic combination of quercetin (as an anti-UV-B agent) and zinc oxide (as an anti-UV-A agent) within a sunscreen lipstick formulation to enhance broad-spectrum photoprotection. A factorial design, implemented using Design Expert 13.0 software, was employed for the optimization. The independent variables were the concentrations of quercetin and zinc oxide, while the dependent responses included pH, Sun Protection Factor (SPF), percentage of Erythema Transmission (%TE), and percentage of Pigmentation Transmission (%TP). Statistical analysis evaluated the individual and interactive effects of the components on these responses. Both quercetin and zinc oxide individually demonstrated a positive influence on increasing the pH and SPF values of the formulations, while concurrently reducing both %TE and %TP values. Notably, a significant synergistic interaction between quercetin and zinc oxide was observed, further influencing the SPF, %TE, and %TP values. The optimized formula, derived from the overlay contour plot generated by the factorial design, comprised 2.5% quercetin and 15% zinc oxide. This optimal combination yielded a pH of 5.027, an SPF of 22.713, a %TE of 0.012, and a %TP of 0.004. The optimized combination of quercetin and zinc oxide in a sunscreen lipstick formulation effectively provided desirable pH values and substantial *in vitro* sunscreen efficacy. These findings suggest that this novel formulation holds significant potential as a broad-spectrum photoprotective cosmetic product.

Keywords:
Factorial design
Quercetin
Sunscreen
Zinc oxide

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INTRODUCTION

The delicate nature of the lips makes them particularly vulnerable to environmental damage. Compared to the rest of the body's skin, lips possess a significantly thinner stratum corneum and notably less melanin, the natural pigment offering protection from ultraviolet (UV) radiation. This reduced endogenous photoprotection leaves the lips highly susceptible to the detrimental effects of UV exposure[1].

Ultraviolet radiation from sunlight is broadly categorized into UVA, UVB, and UVC[2]. While UVC is largely absorbed by the ozone layer, both UVA (90-99%) and UVB (1-10%) reach the Earth's surface[3]. Although UVB radiation plays a crucial

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role in previtamin D synthesis, excessive UV exposure can lead to various adverse skin effects, including erythema, tanning, and localized or systemic immunosuppression[4]. On the lips, prolonged UV exposure can damage protective keratin cells, resulting in uncomfortable chapping[1]. More critically, cumulative UV damage is a primary contributor to lip cancer, with oral squamous cell carcinoma accounting for over 90% of cases affecting the lips[5]. Given these risks, additional photoprotection in the form of sunscreen lipstick is essential, especially under high-intensity UV conditions[6]. An effective sunscreen lipstick must offer comprehensive protection against both UVA and UVB radiation.

To this end, both chemical and physical UV blockers are utilized. Quercetin, a natural chemical absorbent, has demonstrated promising UV-absorbing capabilities[7]; previous research showed that a 3% quercetin formulation provided an Sun Protection Factor (SPF) of 16.11[8]. Concurrently, physical blockers such as zinc oxide are effective in scattering and reflecting UV radiation[9]. For instance, concentrations of 5%, 10%, and 15% zinc oxide yielded SPF values of 4.37, 6.19, and 8.74, respectively, in prior studies[10].

In this study, a creamy (oily) lipstick base was selected for its ability to enhance product longevity on the lips, thereby ensuring sustained UV protection[11]. The overarching goal of this research was to develop an optimized sunscreen lipstick formulation by combining natural chemical compounds with physical blockers. Specifically, we aimed to obtain an ideal sunscreen lipstick preparation and evaluate the synergistic effects of combining quercetin and zinc oxide on crucial formulation parameters, including pH value and sunscreen effectiveness. Efficacy was quantitatively assessed through SPF, which indicates protection against sunburn-causing UV rays; %TE (Erythemal Transmission), representing the percentage of erythema-inducing UV radiation penetrating the lipstick layer; and %TP (Pigmentation Transmission), measuring the percentage of pigmentation-inducing UV rays passing through the lipstick. Lower %TE and %TP values signify superior protection against UV-induced damage[12].

MATERIALS AND METHODS

Materials

The following high-purity chemicals were utilized in this research: quercetin (Tokyo Chemical Industry, Japan), zinc oxide, beeswax, vaseline album, olive oil, oleum cacao, Tween 80, propylene glycol, cocoa butter, Allura Red dye, isopropanol, distilled water, and 96% ethanol. All reagents were of analytical grade and used without further purification. The study employed a range of precision instruments for various experimental procedures. These included suppository molds, an Advanture Ohaus analytical balance for accurate weighing, a Memmert water bath, a Thermo Scientific hot plate, a Genesys 10s UV-Vis spectrophotometer for absorbance measurements, a Denver pH meter, and a GT Sonic ultrasonic cleaner. Standard Pyrex glassware was used for solution preparation and reactions. For particle sizing, a RO-TAP 100-mesh sieve was used, and melting points were determined using a Stuart SMP10 melting point apparatus. Finally, the Design-Expert 13.0 software was utilized for experimental design and data analysis.

Methods

Sunscreen lipstick formulation

The sunscreen lipstick were prepared following formulations, as detailed in **Table I**. Initially, zinc oxide, serving as the physical UV filter, was precisely sieved through a 100-mesh sieve to ensure uniformity and prevent aggregation. Concurrently, the lipid phase components, comprising beeswax, Vaseline album, olive oil, and oleum cacao, were accurately weighed and then melted in a water bath maintained at a temperature range of 70-75°C to achieve a homogeneous molten mixture.

Following the initial melting, this mixture was transferred to a hot plate and continuously stirred at a controlled temperature of 45-50°C to maintain fluidity while preventing degradation of heat-sensitive components. Pure, sieved zinc oxide was then gradually incorporated into this stirred molten base, ensuring uniform dispersion. Subsequently, quercetin, which was pre-dissolved in a minimal volume of ethanol and propylene glycol, was carefully added to the mixture and continuously stirred until a homogeneous solution was achieved. The coloring agent, Allura Red, was then introduced under continuous stirring to ensure even distribution throughout the formulation. Finally, a pre-mixed solution of Tween 80 and olive oil was added to the blend, enhancing emulsification and texture. The resulting molten lipstick mixture was then immediately poured into appropriate molds and allowed to cool and solidify, forming the final lipstick sticks.

Table I. SFCS formula design of ethanol extract of *E. elatior* fruit.

Materials	Function	Quantity (%)			
		$F_{(1)}$	$F_{(a)}$	$F_{(b)}$	$F_{(ab)}$
Quercetin	Active ingredients	0.5	2.5	0.5	2.5
Zinc oxide	Active ingredients	5	5	15	15
Beeswax	Stiffening agent	20	20	20	20
Vaseline album	Base	25	23	15	13
Olive oil	Emollient	13	13	13	13
Oleum cacao	Emollient	2.5	2.5	2.5	2.5
Tween 80	Stabilizing agent	3	3	3	3
Propylene glycol	Cosolvent, humectant	14.5	14.5	14.5	14.5
Allura Red	Dye	1.5	1.5	1.5	1.5
Ethanol 96%	Quercetin solvent	15	15	15	15

Evaluation of sunscreen lipstick

Organoleptic test: The organoleptic properties of the lipstick preparations were assessed through visual observation and sensory evaluation to ensure consistency and quality. Key characteristics such as form, color, texture, and smell were meticulously examined for each formulation. This qualitative assessment provides immediate feedback on the aesthetic appeal and initial user experience of the product, aligning with established cosmetic evaluation protocols[13].

Homogeneity test: Homogeneity of the sunscreen lipstick formulations was assessed by visually inspecting the absence of coarse particles or grittiness. This was achieved by uniformly applying a small amount of the prepared lipstick onto a glass slide and meticulously observing the spread and texture for any discernible irregularities or undissolved components[13].

Smear test: The smear test was conducted to assess the adherence and transfer resistance of the lipstick formulations. Each lipstick preparation was applied five consecutive times to the dorsal aspect of the hand. After a 15-minute equilibration period, the extent of lipstick color remaining on the skin was visually observed and qualitatively evaluated[13].

Melting point test: The melting point of the lipstick preparation was determined using a capillary tube method. Briefly, each sample was packed into a capillary tube to a depth of 10 mm. The filled capillary tube was then carefully placed into a melting point apparatus. The initial temperature of the apparatus was set to 10°C below the anticipated melting point of the preparation. The temperature was subsequently increased at a controlled rate until the sample began to melt, and the temperature at which complete melting occurred was recorded as the melting point[14].

pH test: The pH of the sunscreen lipstick preparation was determined by accurately weighing 1 g of the sample and dispersing it in 10 mL of distilled water. The mixture was gently heated to ensure complete dissolution and uniform dispersion. After allowing the solution to cool to room temperature, a calibrated pH meter was immersed into the mixture, and the pH value was recorded. This procedure was adapted from established methodologies for topical formulations[13].

SPF in vitro: The *in vitro* SPF of the sunscreen lipstick formulations was determined using UV-Vis spectrophotometry. Briefly, a lipstick sample equivalent to 2 mg of active ingredient was accurately weighed and dissolved in 10 mL of 90% ethanol. From this primary solution, 0.5 mL was aliquoted and further diluted to 10 mL with 90% ethanol. The absorbance of this final solution was measured across a wavelength range of 290-320 nm with 1 nm intervals[15]. A key modification was made to the path length of the cuvette. While the original Petro method typically utilizes a 2 cm cuvette, this study employed a 1 cm cuvette. To account for this difference and ensure comparability, the calculated SPF value was multiplied by a factor of 2. The SPF value was subsequently calculated using the following **Equation 1**, where AUC is area under the curve of the absorbance spectrum between λ_1 and λ_n, λ_n is the longest wavelength above 290 nm exhibiting an absorption value greater than 0.05, and λ_1 is the smallest wavelength (290 nm).

$$\text{Log } SPF = \frac{\text{AUC}}{\lambda\text{n}-\lambda 1} \qquad [1]$$

%TE and %TP: The erythema transmission (%TE) and pigmentation transmission (%TP) values of the sunscreen lipstick formulations were determined using a validated UV-Vis spectrophotometric method with isopropanol as the solvent,

adapted from[16]. Briefly, a precise amount of lipstick, equivalent to 2 mg of its active ingredient, was accurately weighed and quantitatively dissolved in 10 mL of isopropanol. A 0.5 mL aliquot of this stock solution was then further diluted to 10 mL with isopropanol to obtain the final test solution. The absorbance of this solution for %TE determination was measured spectrophotometrically at 5 nm intervals across the wavelength range of 292.5 nm to 337.5 nm. Similarly, for %TP determination, absorbance was measured at 5 nm intervals within the wavelength range of 322.5 nm to 372.5 nm. All measurements were conducted against an isopropanol blank. The %TE and %TP values were subsequently calculated using the following **Equations 2** to **4**, where T is transmittance percentage at each specific wavelength, A is absorbance at each specific wavelength, F_e is erythema flux constant at each wavelength, F_p is pigmentation flux constant at each wavelength, $\sum F_e$ is total UV erythema flux across the measured range, and $\sum F_p$ is total UV light pigmentation flux across the measured range.

$$A = -\log T \quad [2]$$

$$\%TE = \frac{\sum(T \times Fe)}{\sum Fe} \quad [3]$$

$$\%TP = \frac{\sum(T \times Fp)}{\sum Fp} \quad [4]$$

Determination and verification of the optimum formula

The optimal sunscreen lipstick formula was determined using Design Expert 13.0 software, aiming for a desirability value approaching 1. This software-guided approach allowed for systematic exploration of formulation variables to identify the most promising combination. To validate the predicted optimal formula, three independent verification batches were prepared. The physicochemical properties and performance of these triplicate batches were then rigorously evaluated and compared against the values predicted by Design Expert 13.0. This comparative analysis served to confirm the accuracy of the software's predictions and ensure the reproducibility of the optimized formulation under experimental conditions.

Data analysis

Statistical analysis was performed using the t-test to determine significant differences between experimental groups. A confidence level of 95% ($p < 0.05$) was maintained for all analyses. A p-value greater than 0.05 indicated that any observed differences in the data were not statistically significant, suggesting that the variations could be attributed to random chance rather than the experimental intervention[17].

RESULTS AND DISCUSSION

The physicochemical properties of all developed lipstick formulations were initially assessed for their organoleptic characteristics (**Table II**). All formulations exhibited consistent form, texture, and scent. A notable distinction emerged in the color intensity (**Figure 1**), which varied across the four formulations. Importantly, none of the formulations left a yellow stain on the skin upon application. As the concentration of quercetin increased, a more pronounced yellow hue was observed. Conversely, increasing the zinc oxide content led to a reduction in the yellow intensity[18].

Table II. Organoleptic test results.

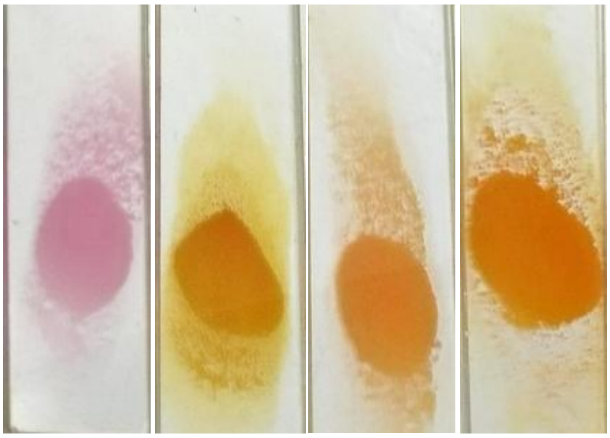
Formula	Form	Color	Texture	Smell
$F_{(1)}$	Semisolid	Pink	Gentle	No smell
$F_{(a)}$	Semisolid	Slightly intense orange	Gentle	No smell
$F_{(b)}$	Semisolid	Orange	Gentle	No smell
$F_{(ab)}$	Semisolid	Coral pink	Gentle	No smell

Further evaluation revealed that all four formulations displayed homogeneous color distribution and were free of coarse particles (**Figure 2**), indicating successful and uniform dispersion of ingredients. The smear test results (**Figure 3**) demonstrated excellent spreadability and consistent color transfer for all formulations. The melting point analysis for all four formulations yielded identical results of 50°C, which falls within the acceptable range for lip preparations (50-70°C). This consistent melting point is primarily attributed to the standardized amounts of beeswax and cocoa butter in all formulations.

Cocoa butter, with its relatively low melting point (35-40°C), contributes to the emollient properties, while beeswax (melting point 62-65°C) plays a crucial role in elevating and stabilizing the overall melting point of the lipstick[19,20].

a **b** **c** **d**

Figure 1. Sunscreen lipstick formulation results: (**a**) $F_{(1)}$; (**b**) $F_{(a)}$; (**c**) $F_{(b)}$; and (**d**) $F_{(ab)}$.



a **b** **c** **d**

Figure 2. Homogeneity test of lipstick sunscreen results: (**a**) $F_{(1)}$; (**b**) $F_{(a)}$; (**c**) $F_{(b)}$; and (**d**) $F_{(ab)}$.

a **b** **c** **d**

Figure 3. Smear test of lipstick sunscreen results: (**a**) $F_{(1)}$; (**b**) $F_{(a)}$; (**c**) $F_{(b)}$; and (**d**) $F_{(ab)}$.

Regarding pH values (**Table III**), $F_{(ab)}$ recorded the highest pH at 5.03, while $F_{(1)}$ had the lowest at 4.37. All four formulations met the established pH criteria for topical lip preparations (4.0-6.5) and the stability requirements for quercetin (pH 1-6)[19,21]. Factor effect calculations and variance analysis (**Figure 4**) indicated that both quercetin and zinc oxide significantly influence pH levels, with increasing concentrations leading to higher pH values. This is likely due to the ionization reaction of quercetin molecules in the pH range of 4 to 6[22], and the inherent alkaline nature of zinc oxide (pH 8.13).

The SPF values for the four formulations varied, with $F_{(1)}$ categorized as "maximum protection," while $F_{(a)}$, $F_{(b)}$, and $F_{(ab)}$ fell into the "ultra protection" category[23]. $F_{(ab)}$ exhibited the highest SPF value at 22.71, whereas $F_{(1)}$ had the lowest at 13.21.

Statistical analysis confirmed that quercetin, zinc oxide, and their synergistic interaction significantly increased SPF values. This can be attributed to quercetin's chromophore group, which effectively absorbs UV light, and zinc oxide's established role as an inorganic UV filter that provides broad-spectrum protection by absorbing UV radiation[24].

Further assessment of sunscreen effectiveness was conducted through %TE and %TP values. All four formulations demonstrated values below 1, classifying them within the "total block" category[23]. $F_{(ab)}$ exhibited the lowest %TE (0.12426) and %TP (0.004153) values, indicating superior blocking capabilities, while $F_{(1)}$ showed the highest %TE (0.563520) and %TP (0.515975). Factor effect calculations and variance analysis revealed that both quercetin and zinc oxide significantly reduced %TE and %TP values, suggesting their individual contributions to UV protection. Conversely, the interaction between quercetin and zinc oxide significantly increased %TE and %TP values, which warrants further investigation to understand this complex interplay. Specifically, quercetin is effective in absorbing UVB rays, thereby reducing %TE[7]. Zinc oxide also contributes to %TE reduction through its ability to absorb a small amount of UVB. For %TP, quercetin can absorb a small amount of UVA light, and zinc oxide is known as an effective anti-UVA agent[25].

Table III. Test results.

Formula	pH	SPF	%TE	%TP
$F_{(1)}$	4.37 ± 0.044	13.21 ± 0.185	0.563520 ± 0.006934	0.515975 ± 0.006934
$F_{(a)}$	4.61 ± 0.031	16.24 ± 0.047	0.355403 ± 0.007024	0.338402 ± 0.007705
$F_{(b)}$	4.88 ± 0.04	19.04 ± 0.031	0.069942 ± 0.005528	0.041492 ± 0.000941
$F_{(ab)}$	5.03 ± 0.042	22.71 ± 0.085	0.012426 ± 0.002083	0.004153 ± 0.000450

Note: Data are presented as mean ± standard deviation (n=3)

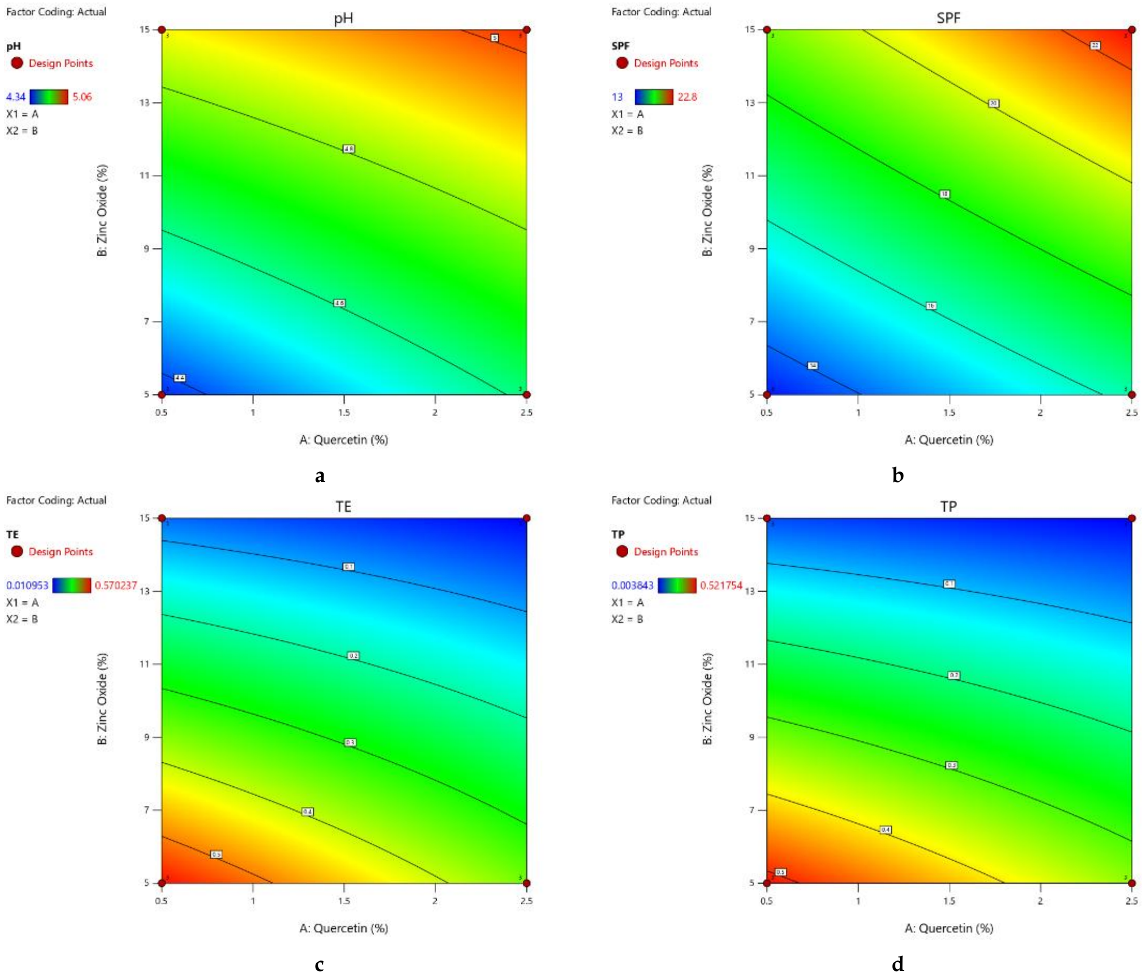


Figure 4. Contour plot of response: (**a**) pH; (**b**) SPF; (**c**) %TE; and (**d**) %TP.

Based on these comprehensive analyses, the optimal lipstick formulation was identified as $F_{(ab)}$, containing 2.5% quercetin and 15% zinc oxide, with a desirability value of 0.924. This optimal formulation yielded predicted responses of pH 5.027, SPF 22.713, %TE 0.012, and %TP 0.004. Verification results for the optimal formula's pH, SPF, %TE, and %TP values showed no statistically significant differences (p >0.05) when evaluated using a t-test at a 95% confidence level[26]. This outcome confirms that the optimization design model, utilizing Design Expert 13.0 software, accurately predicted the experimental responses. Consequently, the optimal formula effectively met the desired physicochemical and sunscreen efficacy requirements for the developed lipstick.

CONCLUSION

This study demonstrates that the concentrations of both quercetin and zinc oxide significantly influence the SPF, as well as %TE and %TP, of lipstick sunscreen formulations. Higher concentrations of these active ingredients were found to enhance the SPF value while simultaneously reducing %TE and %TP, indicating improved sun protection. Interestingly, a synergistic interaction between quercetin and zinc oxide was observed, further boosting the SPF value, %TE, and %TP of the formulations. Furthermore, increasing the amounts of these compounds also led to a discernible increase in the pH of the lipstick sunscreen formula. The optimized formulation, specifically the one containing 2.5% quercetin and 15% zinc oxide, exhibited favorable pH values and demonstrated promising *in vitro* sunscreen effectiveness, suggesting its potential as an effective photoprotective cosmetic product.

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AUTHORS' CONTRIBUTION

Conceptualization: Budipratiwi Wisudyaningsih, Kuni Zu'aimah Barikah
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Investigation: Budipratiwi Wisudyaningsih, Ilfi Nur Kamelia
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Resources: -
Software: -
Supervision: Budipratiwi Wisudyaningsih, Kuni Zu'aimah Barikah
Validation: Budipratiwi Wisudyaningsih, Kuni Zu'aimah Barikah
Visualization: Kuni Zu'aimah Barikah, Ilfi Nur Kamelia
Writing - original draft: Kuni Zu'aimah Barikah, Ilfi Nur Kamelia
Writing - review & editing: Budipratiwi Wisudyaningsih

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this study.

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