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Influence of Solvent Polarity on Color Intensity of Marigold Flower Extracts

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Abstract. Marigold (*Tagetes erecta* L.) flowers contain carotenoids as the primary pigments and flavonoid/phenolic compounds as color-contributing constituents responsible for their yellow to orange coloration. This study aimed to determine the effect of solvent polarity on extraction efficiency, spectral profile, stability and color intensity of marigold flower extracts. Yellow marigold (YM) and orange marigold (OM) flower powders were extracted by maceration using ethyl acetate (EA) and n-hexane (HX) to obtain dried extracts. The extraction results showed that YM extracted with EA produced a higher yield than YM extracted with HX, whereas OM extracted with HX produced a higher yield than OM extracted with EA. UV–Vis spectroscopic analysis was conducted to characterize the dried extracts, and all samples exhibited similar spectral profiles with absorption bands corresponding to carotenoids and flavonoid/phenolic compounds. Extract stability was evaluated by monitoring shifts in λ max values over a 30-day storage period, and all dried extracts demonstrated stable spectral profiles. Color intensity analysis using a color reader based on the CIE L*a*b* system revealed that EA extracts exhibited higher color intensity than HX extracts for both marigold flower colors. Overall, solvent polarity significantly influenced extraction yield and color intensity, while the chemical stability of the dried extracts remained unaffected during storage.

Keywords : Natural dye, *Tagetes erecta* L., solvent polarity, color intensity, maceration.

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Introduction

Research on the isolation and constituent components of natural dyes from plants is relatively growing. This is due to the fact that natural dyes are widely used in the food and health supplement industries as they have attractive colors and are safe for consumption, so they can minimize the occurrence of diseases caused by synthetic dyes [1]. In addition, consumer demand for “clean-label” products and environmentally friendly processing has further driven interest in plant-based pigments [2].

One plant with a natural yellow and orange color is the marigold flower. The color comes from carotenoid, tetraterpene compounds that built primarily from multiple isoprene units. Their carbon backbone typically contains nine conjugated double bonds, with terminal end groups at both ends of the molecule [3,4]. The pigments responsible for the color in marigold petals include lutein and other carotenoid such as zeaxanthin and β-carotene [5,6]. A study also reported that the greater the number of conjugated double bonds, the higher the absorption maxima (λ max). As a result, the color of carotenoids ranges from yellow, red to orange in many plants [7].

Carotenoids are lipophilic and insoluble in water but are soluble in various organic solvents such as acetone, ethanol, ethyl ether, chloroform, and ethyl acetate. The choice of solvent influences extraction efficiency, with non-polar solvents like n-hexane favoring the recovery of non-polar carotenes, and moderately polar solvents such as ethyl acetate facilitating the extraction of polar xanthophylls [4,8]. Consequently, carotenoids from marigold petals are commonly extracted using Soxhlet, maceration, microwave-assisted extraction methods or ultrasound-assisted extraction methods with appropriate solvent systems [9, 10].

However, carotenoids are chemically unstable due to their highly unsaturated structure, making them susceptible to oxidation, photo-degradation, and isomerization when exposed to heat, light, oxygen, or acids. These degradation processes can lead to significant color loss and reduced bioactivity, which is why stability studies are essential during both extraction and storage phases [4,11].

Every natural dye has a distinctive color profile based on the characteristics of the compound. The assessment of color quality and stability can be conducted using analytical techniques such as UV-Vis spectrophotometry, which provides information on absorption spectra, and colorimetry, which quantifies color in the CIE L*a*b* color space. In this study, UV-Vis spectrophotometry was applied to characterize the spectral properties of yellow and orange marigold extracts, while a color reader objectively measured color intensity and chromaticity parameters.

Accordingly, the objectives of this research are to optimize the extraction process for maximum yield and color intensity, evaluate the stability of the extracted pigments under different conditions, and characterize the colorimetric profiles of marigold flower petal extracts to support their potential application as safe, natural colorants in the food and health supplement industries.

Experimental

Materials and Tools. Materials used in this research are yellow and orange marigold flowers (*Tagetes erecta* L.) obtained from farmers in Bumiaji Village, Batu (7°50'09.4"S 112°33'27.2"E), and the petals processed into powder at the UPT Lab. Herbal Materia Medica Batu. Solvents used were n-hexane and ethyl acetate (re-distilled), nitrogen gas, and analytical grade solvents from SMART-LAB.

Equipment included standard glassware (Pyrex), analytical balance (Sartorius BSA224S-CW), vortex mixer (Oregon), and centrifuge (Oregon LC-04S). Colorimetry analysis was carried out using a color reader ColorFlex EZ0530.

Plant Determination. Plant determination was conducted at the UPT Herbal Laboratory of Materia Medica Batu by comparing morphological characteristics with standard identification keys.

Marigold Flower Powder Preparation. Marigold flower powder preparation carried out by harvesting fresh marigold flowers at the full-bloom stage in the morning, followed by sorting and thorough washing of the petals with running water. The cleaned petals were oven-dried at 50 °C, ground using a grinder, and sieved through a 90-mesh sieve to obtain a fine powder. Powder preparation was conducted at the UPT Herbal Laboratory of Materia Medica Batu.

Table 1. Extracts code based on marigold flower color and solvent used for extraction

Extracts Code	Marigold Flower Color	Solvent
YM-HX	Yellow	n-Hexane
YM-EA	Yellow	Ethyl acetate
OM-HX	Orange	n-Hexane
OM-EA	Orange	Ethyl acetate

Marigold Flowers Extraction with the Maceration Method. Marigold flowers extraction with the maceration method was performed by macerating 100 g of marigold powder with 1000 mL of n-hexane and ethyl acetate in separate Erlenmeyer flasks for 2 × 24 h. The macerate was then filtered to separate the filtrate (extract) from the residue. The extracts were concentrated using a rotary evaporator and subsequently dried under a stream of nitrogen gas until a constant mass was achieved, then collected and stored in dark airtight containers for further analysis. The extracts code based on marigold flower color and extraction solvent are presented in **Table 1.**

UV-Vis Spectrum Profile Analysis of Marigold Flower Dried Extracts. UV–Vis spectrum profile analysis of marigold flower dried extracts was performed by diluting the dried extracts to a concentration of 0.1 mg/mL in n-hexane. Then the extract solution was analyzed with a UV-Vis spectrophotometer at a wavelength range of 200–600 nm.

Stability Study of Marigold Flower Dried Extracts. The stability of the dried extracts was observed using a UV-Vis spectrophotometer during 30 days of storage inside a dark container under room temperature. Stability was evaluated by observing any shifts in five absorption maxima (λ max) values, two corresponding to flavonoid/phenolic bands and three to the carotenoid spectral fine structure at 200–600 nm region of the UV–Vis spectra. UV-Vis analysis was carried out on days 0, 1, 3, 5, 10, 20, and 30. The measurements were conducted to evaluate extracts stability during storage and to assess whether the extracts retained observable characteristics during this period.

Analysis of The Color Intensity of Marigold Flower Extracts. Analysis of the color intensity of marigold flower extracts was performed using a ColorFlex EZ0530 color reader to measure CIE L*, a*, and b* values in the visible wavelength range (400–700 nm) at the Pharmacy Laboratory of Ma Chung University, Malang.

Result and Discussion

Plant Determination. Plant determination confirmed the species classification of the sample as Tagetes erecta L., with representative photographs of the marigold plant presented in **Figure 1.**

Marigold Flower Powder Preparation. Marigold flower powder preparation successfully produced dried powders from both orange and yellow marigold flowers. The dried powders were subsequently characterized and quantified, as summarized in **Table 2.** The moisture content of the yellow marigold powder and orange marigold powder was found to be 2.9% and 3.5%. These values indicate that both powders meet the quality requirements for dried herbal materials, as moisture contents below 10% are generally considered acceptable. Low moisture content is essential to minimize microbial growth, enhance storage stability, and prevent degradation of bioactive compounds [12]. Therefore,

Figure 1. Yellow and orange marigold flowers

Table 2. Characteristics of marigold flower powders obtained from preparation

Marigold Flower Color	Flower Mass (g)		Yield	Moisture Content
	Fresh	Dried	(%)	(%)
Yellow	2.03	0.32	15.8	2.9
Orange	2.2	0.3	13.6	3.5

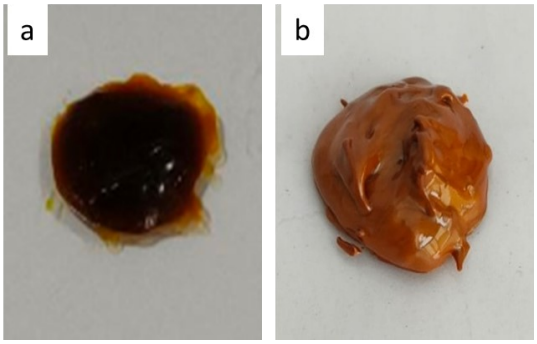

Figure 2. Marigold dried extract obtained from (a) yellow marigold and (b) orange marigold

the obtained marigold powders can be regarded as suitable raw materials for subsequent extraction processes.

Marigold Flowers Extraction with the Maceration Method. Marigold flowers extraction with the maceration method produced dried extracts after solvent reduction, as shown in **Figure 2.** The orange marigold dried extract showed a lighter color compared to the yellow marigold dried extract. The dried extracts were weighed to determine the extraction yield for each treatment. The yields obtained using n-hexane and ethyl acetate solvents are presented in **Table 3.**

The findings from this study indicate that the polarity of the solvent used had an impact on the yield of both yellow and orange marigold flower dried extracts. Specifically, the highest yield was achieved when utilizing n-hexane as the solvent for extracting orange marigold petals. Conversely, when extracting yellow marigold flowers, the highest yield was attained through the use of ethyl acetate as the solvent.

Marigold flowers are known to be rich in carotenoids, particularly lutein esters, which represent the dominant carotenoid fraction [13]. Lutein esters exhibit non-polar characteristics, resulting in higher solubility in non-polar solvents such as n-hexane [14]. In this study, the n-hexane extract yield of orange marigold (OM-HX) was higher than that of yellow marigold (YM-HX). This difference can be attributed to the higher lutein ester content in marigold flowers with deeper orange to reddish coloration, as color intensity is closely associated with carotenoid accumulation [15].

In addition to carotenoids, marigold flowers also contain phenolic compounds and flavonoids such as quercetin and kaempferol [16]. These compounds are generally polar to semi-polar in nature and therefore exhibit higher solubility in polar or semi-polar solvents, such as ethyl acetate. In the present study, the ethyl acetate dried extract yield of yellow marigold (YM-EA) was higher than that of orange marigold (OM-EA). Although orange marigold is known to contain higher carotenoid levels, the greater extraction yield observed for YM-EA suggests that yellow marigold flowers may possess higher concentrations of extractable phenolic and flavonoid compounds. This result is in line with previous studies reporting that yellow marigold flowers (Arka Bangara) possess higher total flavonoid content (TFC) and total phenolic content (TPC) than orange marigold flowers (Arka Agni), supporting the assumption that the higher ethyl acetate extract yield of yellow marigold is associated with a greater abundance of polar phenolic and flavonoid compounds [16].

UV-Vis Spectrum Profile Analysis of Marigold Flower Dried Extract. The UV-Vis spectrum profiles of marigold flowers dried extracts in n-hexane solvent observed at a wavelength of 200-600 nm are shown in **Figure 3.**

UV–Vis spectra of the four dried extracts exhibited similar spectral profiles, with five λ max values consistently observed in each extract, although differences in absorbance intensity were detected. Three λ max values were observed in the 400–500 nm region and two λ max value were observed in the 255-350 nm region as shown in Figure

Table 3. Extraction results of marigold flowers

Dried Extracts*	Extract mass (g)	Yield (%)	Physical Properties of Marigold Dried Extracts**	
			Color	Consistency
YM-HX	8.6682	8.6	Dark Brown	Oily Paste
YM-EA	14.8044	14.8	Dark Brown	Oily Paste
OM-HX	9.6184	9.6	Light Brown	Oily Paste
OM-EA	8.0357	8	Light Brown	Oily Paste

* Refer to **Table 1**

** The dried extracts were observed after reaching a constant mass, confirming that no residual water or solvent remained.

3. The λ max at 400-500 nm region correspond to carotenoid pigments, which exhibit tri-peaked spectral fine structure in the blue–violet region due to their conjugated π-bond system, resulting in yellow-to-red coloration [7,11,17]. Consistent with a previous study reporting that lutein accounts for 88–97% of total carotenoids in marigold flower extracts, this spectral pattern confirms the presence of carotenoids in all extracts regardless of flower color or extraction solvent. The UV–Vis spectra also showed two λ max in the 255–350 nm region, which are commonly associated with flavonoid and phenolic compounds, namely band I (310-350 nm) and band II (255-280 nm) [18]. This suggest that the extracts also contain flavonoid and phenolic compound.

Variations in absorbance are attributed to differences in the concentration of bioactive compounds within each extract, which is consistent with the Lambert–Beer law. Accordingly, higher absorbance values correspond to higher concentrations of light-absorbing pigments, and the observed differences in color intensity among the extracts are directly related to variations in pigment concentration [19].

Stability Study of Marigold Flower Dried Extracts. The stability of marigold flower dried extracts was evaluated by observing shifts in five λ max values in the UV–Vis spectral profiles. The stability profiles of the five λ max values for the marigold flower dried extracts are presented in **Figure 4.**

The λ max of carotenoid spectral fine structure denoted as λ_1, λ_2, and λ_3. The λ max of flavonoid/phenolic profile bands denoted as λ4 and λ5. Based on Figure 4, minor shifts were observed at λ4 and λ5, with wavelength differences within a range of 2–3 nm. The stability evaluation indicated that the λ max values corresponding to both carotenoid and flavonoid/phenolic compounds remained largely stable during the 30-day storage period. Minor shifts were only observed at λ4 and λ5, which are associated with flavonoid/phenolic bands. However, the wavelength changes were limited to 2–3 nm and fell within the margin of error, indicating no significant spectral alteration. The consistent λ max profiles across all dried extract solutions suggest that the chromophoric systems of both compound classes were preserved, with flavonoid/phenolic components exhibiting particularly high spectral stability. Overall, these findings confirm that the marigold dried extracts maintained their UV–Vis

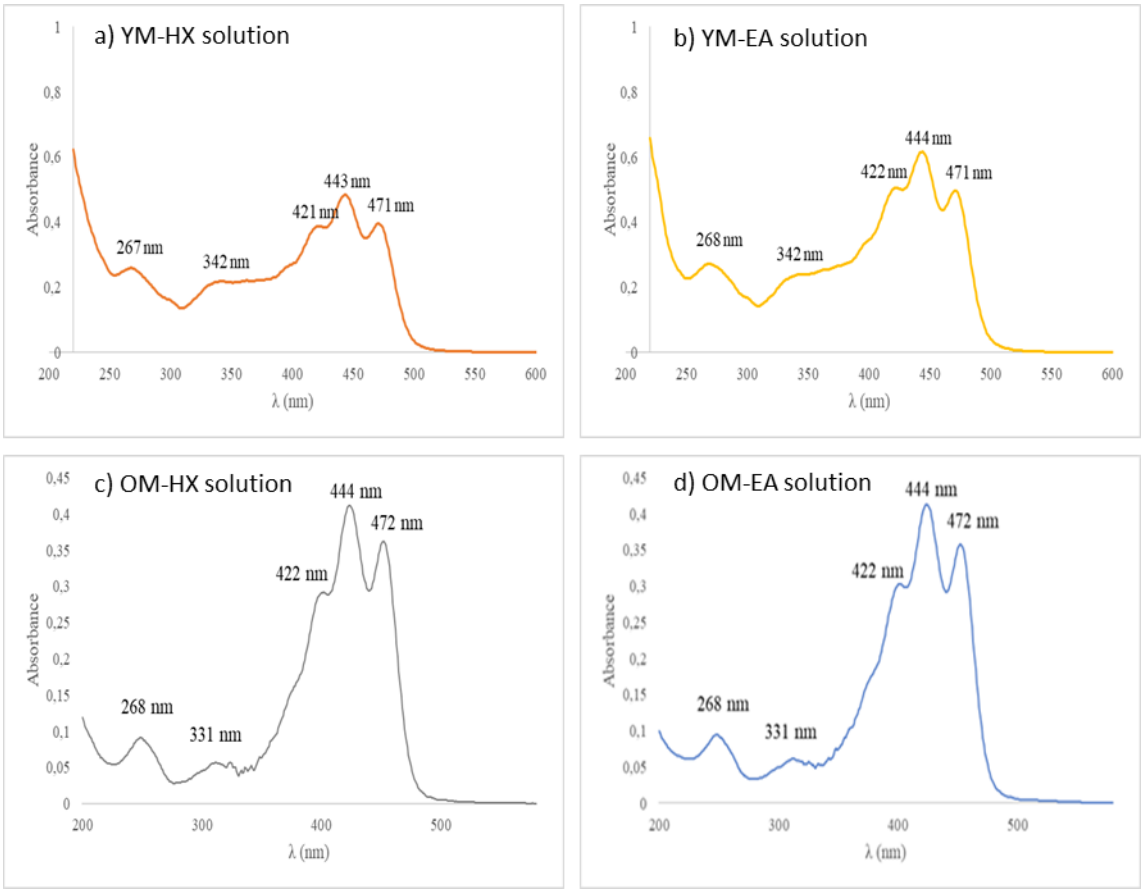

Figure 3. UV-vis spectra profile of diluted marigold flower extracts in n-hexane (0.1 mg/mL)

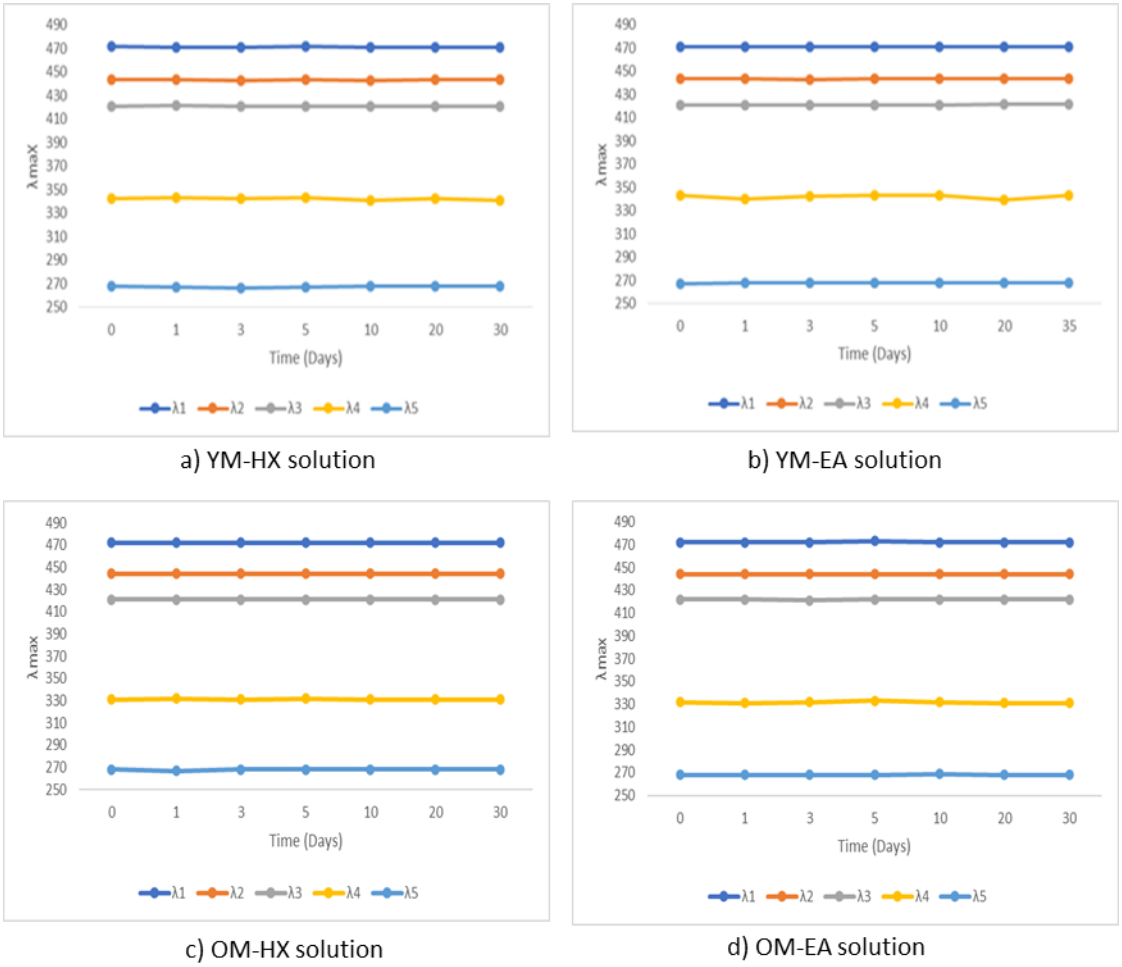

Figure 4. Stability profiles of marigold flower dried extracts based on λ max values during 30 days of storage

spectral integrity over the storage period, supporting their suitability for further analytical and formulation studies.

Analysis of the Color Intensity of Marigold Flower Extracts. Analysis of the color intensity of marigold flower extracts quantitatively evaluated CIE L*, a*, and b* values of extracts obtained by maceration. This analysis was performed to compare color characteristics across different flower colors and extraction solvents. The analyzed extracts are shown in **Figure 5,** while the corresponding color intensity results are presented in **Table 4**.

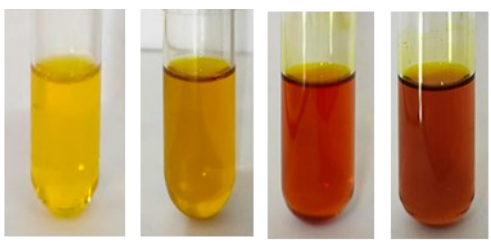

Figure 5. Extracts of yellow (a, b) and orange (c, d) marigold flowers

The CIE L*, a*, and b* parameters were used to describe the color characteristics of the samples. The L* value indicates lightness (0 = black, 100 = white), a* represents the red (+) to green (−) axis, and b* represents the yellow (+) to blue (−) axis. Variations in L*, a*, and b* values can reflect differences in pigment composition, such as carotenoids and flavonoids, in the flower extracts [16].

Based on **Table 4**, ethyl acetate (EA) extracts showed lower lightness (L*) values than n-hexane (HX) extracts for both marigold flower colors. This result indicates a darker color of EA extracts, possibly due to the higher solubility of polar pigments in ethyl acetate. Orange marigold (OM) extracts exhibited higher a* values, reflecting a redder hue associated with higher carotenoid content. This observation is consistent with previous report that darker marigold flowers contain higher carotenoid levels, which contribute to orange–red coloration [15]. All extracts exhibited positive b* values, suggesting a predominant yellow coloration. Ac-

Table 4. L*, a* and b* Color Intensity Coordinates of *Marigold Flower Extract*

Extracts*	CIE parameters			Color Conversion**	Visual Appearance
	L*	a*	b*		
YM-HX	71.62	-10.75	63.23		
YM-EA	68.40	-6.65	86.86		
OM-HX	63.57	2.89	99.07		
OM-EA	54.26	24.55	90.91		

* Refer to **Table 1**.

** Conversion of CIE Lab values to hexadecimal (HEX) color codes was performed using an online color conversion tool

cording to opponent-color theory, absorption in the blue region around 444 nm results in the perception of its complementary color, namely yellow to orange [20]. Therefore, the UV–Vis absorption profiles observed in the 420–475 nm region are in good agreement with the yellow and orange hues identified in the color intensity analysis.

Conclusion

Solvent polarity significantly influenced the extraction yield and color intensity of marigold flower extracts. Ethyl acetate produced higher yields for yellow marigold dried extracts (14.8 %), whereas n-hexane was more effective for extracting orange marigold (9.6 %). UV–Vis characterization confirmed that all dried extracts contained carotenoid and flavonoid/phenolic compounds with similar spectral profiles. Stability evaluation based on periodic λ max value indicated that the dried extracts remained stable over 30 days of storage for every dried extracts. Color intensity analysis showed that ethyl acetate generated extracts with higher color intensity than n-hexane for both marigold flower colors. These findings indicate that ethyl acetate is a more efficient solvent for producing intensely colored marigold extracts, while both solvents are suitable for obtaining stable dried extracts.

Author Contributions

R. R., H. S., S. M. U., B. K. and S. designed and supervised the project. H. S. and B. K. contributed to the development of the methodology. I. A. D., S. P. M., and R. R. A. helped with the experiments, collected the data, and conducted the data analysis. R. R., H. S., S., and S. M. U. provided guidance on data interpretation. S. P. M., I. A. D., and R. R. A. drafted the manuscript, and R. R. and R. R. A. reviewed and edited the final version.

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