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INNOVATIVE AND COST-EFFECTIVE SESZEN-BIO™ WITH ENRICHED BIOTIN AND IMPROVED SUPERIOR DISSOLUTION EFFICIENCY

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ABSTRACT

Background: Micronutrient deficiencies, including biotin, iron, zinc, and vitamin D, can lead to hair problems such as loss and thinning. This study explores the improvement of SesZen-Bio™, a natural biotin supplement derived from *Sesbania grandiflora* leaves, to give NI- SesZen-Bio™. **Materials:** The nature and structure of biotin in SesZen-Bio™ were evaluated using C14 analysis and ^{1}H NMR. Further, *Sesbania grandiflora* was subjected to an improved economic process to give NI-SesZen-Bio™. HPLTC-aided phytochemical profiling was performed for SesZen-Bio™, NI- SesZen-Bio™, and standard *S. grandiflora* leaf extract. The purity of d-biotin was quantified using HPLC. The dissolution profile of SesZen-Bio™ and NI- SesZen-Bio™ was tested to determine bioequivalence and compared versus synthetic biotin. **Results:** NMR and C14 analysis revealed that SesZen-Bio™ contained 100% natural d-biotin. The yield for SesZen-Bio™ and NI- SesZen-Bio™ was found to be 17% and 18%, respectively, and both exhibited phytochemical profiles similar to standard extract. The release rate for SesZen-Bio™ was similar till a dose of 500 mg and slowly decreased at higher concentrations (800-100mg). A 350 mg NI- SesZen-Bio™ showed bioequivalence to 250 mg and 500 mg for SesZen-Bio™. Percent d-biotin content was 0.5% in SesZen-Bio™ and 0.81% in NI- SesZen-Bio™. **Conclusion:** This new economically driven process yielded NI-SesZen-Bio™, with similar yield, higher biotin (100%natural) content, and similar dissolution profile but at a reduced dosage as compared to SesZen-Bio™ and surpassed synthetic biotin with its sustained release format. Thus, NI- SesZen-Bio™ is a promising solution for individuals seeking natural supplements for maintaining hair and skin heath.

INTRODUCTION

Apart from chronological aging, hereditary reasons, and autoimmune diseases, other factors responsible for hair damage include stress, lifestyle, infections, and lack of appropriate nutrients [1]. A deficiency of micronutrients, including minerals (selenium, copper, iron, and zinc), vitamins (A, D, E, K, and B-complex), and essential fatty acids in the human body can result in several hair, skin, and nail cutaneous abnormalities [2]. One of the major vitamins required by the human body is Vitamin B7 or biotin. Vitamin B7 or Biotin is not synthesized by mammals, and humans are dependent on dietary intake from plant sources.

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Certainly, biotin is produced in the body by microbial populations [3]. Biotin coenzyme assists in fatty acid synthesis, breakdown of branched-chain amino acids, and gluconeogenesis; these pathways play a key role in maintaining skin and hair health [4]. The biotin deficiency can lead to problems from alopecia, skin infections, and conjunctivitis to severe abnormalities such as developmental delay in infants, ataxia, acidosis, and seizures [3].

Biotin supplementation is a gold marketing strategy for skin care and hair growth products. The intake of biotin in the US population is approximately 30 mcg/day, with no upper limit for biotin consumption [5]. Several reports suggest that even higher dosages of 600 times the normal dietary intake have shown gene variations, but the reports do not specify if the changes are undesirable [6]. Synthetic and food-derived vitamins are available in the market. Though they are chemically identical, certain other phytocompounds in natural vitamin origins, such as phytochemicals, minerals, and dietary fibers, can affect the bioavailability of such vitamins [7]. On the other hand, micronutrients can also increase the bioavailability of other phytocompounds. For instance, vitamin C is known to increase the bioavailability of polyphenols and bioflavonoids and has been known to modulate the biological activities of quercetin and other polyphenols [7]. Also, in another study, lactating women were supplemented with natural and synthetic α-tocopherol and compared against the baseline group who received neither natural nor synthetic supplements. The increase in the α-tocopherol in colostrum was measured, and it was found that there was an increase in the levels upon supplementation. However, natural α-tocopherol supplementation increases the percentage of α-tocopherol in the colostrum by 57% compared to a 39% increase in women supplemented with synthetic α-tocopherol [8].

In another study conducted by Lindschinger *et al*. (2020), a comparison was made between the effects of natural and synthetic vitamin B complex supplements on 30 volunteers. Fifteen participants received natural vitamin B complex (Panmol B Complex derived from Quinoa sprouts), while the other 15 received the synthetic counterpart. Although the study did not find a statistically significant difference in the bioavailability of the two groups, noteworthy outcomes were observed. Despite the lack of significant differences in bioavailability, the levels of natural vitamins increased and remained elevated even after the supplementation period ended and continued into the washout period. Additionally, participants who received natural vitamins exhibited better oxidative stress regulation than those who received synthetic counterparts. This suggests that while natural and synthetic supplements may have similar bioavailability, natural vitamins may offer additional benefits, including sustained elevated levels and improved regulation of oxidative stress [9]. Also, the presence of other phytochemical cofactors in the extract enriched for natural biotin might have several other benefits attributed to enhanced absorption.

As mentioned before, natural alternatives to biotin (other vitamins) could benefit over their synthetic counterparts, such as increased bioavailability, other important bioactive phytochemicals, and increased sustenance post-supplementation. A product SesZen-Bio™, a *Sesbania grandiflora* extract from leaves, standardized for biotin along with gallic acid equivalent polyphenols can thus prove beneficial. The product SesZen-Bio™ can be used as a natural biotin supplement. It was also tested for hair and skin benefits in a CTRI registered trial (CTRI/2022/10/046324) [10], with 250 mg of the extract of Sesbania grandiflora leaves standardized to 0.5% biotin administered twice orally. In the current study, SesZen-Bio™ was also authenticated for the presence of synthetic biotin. Further, the extraction method was improved to give an upgraded version of SesZen-Bio™ with higher biotin content. This improvised version of SesZen-Bio™, i.e., New Improved SesZen-Bio™ (NI- SesZen-Bio™), was developed to address sustainability concerns in the natural ingredients industry as lower energy consumption gave a better product dynamic. The old and NI- SesZen-Bio™ were tested for percent biotin content and dissolution profile compared to synthetic biotin. This NI- SesZen-Bio™ had higher biotin content and better-sustained dissolution in comparison to SesZen-Bio™ as well as synthetic biotin, which may have several advantages, including better product performance, economics, and competitive edge over other products that are consumed at higher dosages to deliver the same content of bioactive biotin.

To the best of our knowledge, we are among the few to study the extraction of high-purity natural biotin extract, its characterization, and dissolution compared to synthetic biotin. The aim is to provide an improved, standardized, and concentrated natural biotin supplement that addresses nutritional deficiencies and supports holistic hair and skin health at a reduced dose.

MATERIALS AND METHODS

All the materials used for extraction were analytical grade, whereas all the chemicals used for sophisticated instrumentation analysis were high-purity.

1H NMR and Radiocarbon C14 analysis

The 1H NMR analysis was done at the Shraddha Analytical lab, and the C14 analysis was conducted at the Beta Analytical testing laboratory for SesZen-Bio™.

Extraction of SesZen-Bio™

The leaves were subjected to extraction in hydroalcoholic solution for 4 hours and 6 hours at 50-60°C in 90% ethanol. This step was repeated three times. The extract was further subjected to vacuum tray drying to obtain *Sesbania* powder extracts. The standardized extract was labeled as SesZen-Bio™ when extracted for 6 hours and NI-SesZen-Bio™ with reduced extraction time.

HPTLC fingerprinting

The HPTLC fingerprinting was conducted by Tamilnadu Test House, Tamil Nadu. *Sesbania grandiflora* extract (internal reference standard), SesZen-Bio™, and NI- SesZen-Bio™ were compared for their biochemical profile using high-performance thin-layer chromatography (HPTLC) techniques. The mobile phase employed to separate the band was chloroform: methanol (8:2). The separation was carried out on a silica gel HPTLC plate, and the bands were detected at 254 and 366 nms.

Phytochemical release profile

The amount of biotin released from SesZen-Bio™, NI- SesZen-Bio™, and synthetic biotin was evaluated for 4 hours in media replicating intestinal juice at pH 6.8 using the dissolution apparatus (Electrolab). The study aimed to investigate the variations in the release pattern of SesZen-Bio™ at four different concentrations (250mg, 500mg, 800mg, and 1000 mg) using an *in-vitro* dissolution profiling approach. For NI-SesZen-Bio™, the concentrations chosen were 350 and 500 mg, whereas synthetic biotin was tested at 100 and 250mg.

The experimental design involved mimicking gastrointestinal conditions with pH 6.8 media. The intestinal juice was prepared as per the methodology given by Patricio *et al*., 2012. The dissolution tests were conducted at 37°± 0.5°C with a rotation speed of 50 RPM to replicate body temperature and physiological conditions. Sampling was performed at key time intervals of 60, 120, 240, and 360 minutes, and the collected samples were subjected to content analysis using High Performance Liquid Chromatography (HPLC). The HPLC conditions were optimized to quantify biotin accurately. The study included statistical analysis to compare release patterns at different concentrations and time points. The percent release was used to determine the similarity factor (f2) between the different product concentrations and the two products [11]. A value between 50-100 is considered a similar dissolution profile.

HPLC analysis

The analysis method was adapted from AOAC Official Method 2016.02 to detect Biotin in infant formula and adult/pediatric nutritional formulas [12]. The analysis was conducted by Als Testing Services India Pvt Ltd on a 4.6 mm x 15 cm, 3µm packing L7 column, and the flow rate used was 1.2 ml/min.

RESULTS

1H NMR and Radiocarbon C14 analysis

As per the 1H NMR analysis (Figure 1), the structure of biotin derived from standardized SesZen-Bio™ was markedly different from that of synthetic molecules in terms of cyclic and aliphatic chains. The cyclic ring was identified, while the aliphatic CH_2 groups were not showing in the 1H NMR spectrum for SesZen-Bio™. Hence, natural biotin has lower molecular complexity, which might lead to better permeability, which is expected in plant-based biotin. Additionally, as per the radioactive C14 analysis, it was seen that 100% of the carbon present in SesZen-Bio™ belongs to the natural environment and is not of synthetic origin.

Standardization and extraction of SesZen-Bio™ and NI-SesZenBio™

The yield of NI-SesZen-Bio™ was 18% with a 4-hour extraction in 90% ethanol, as compared to 17% for SesZen-Bio™, which was extracted in 90% ethanol for 6 hours. Thus, by reducing the solute-solvent interaction time at 60°C, a better yield was obtained for NI-SesZen-Bio™.

HPTLC fingerprinting

Upon confirmation of the nature and structure of natural biotin present in SesZen-Bio™, the extraction process was further altered to give more purity of biotin to give NI- SesZen-Bio™. The HPTLC technique was employed to authenticate and

compare the phytochemical contents of reference standard *S. grandiflora* leaf extract with SesZen-Bio™ and NI- SesZen-Bio™. From Figures 2 and 3, it was confirmed that both SesZen-Bio™ and NI- SesZen-Bio™ showed a band pattern that matched with the *S. grandiflora* leaf extract (internal reference standard). Further, the HPLC analysis was conducted to determine the percent of biotin content present in SesZen-Bio™ and NI-SesZenBio™

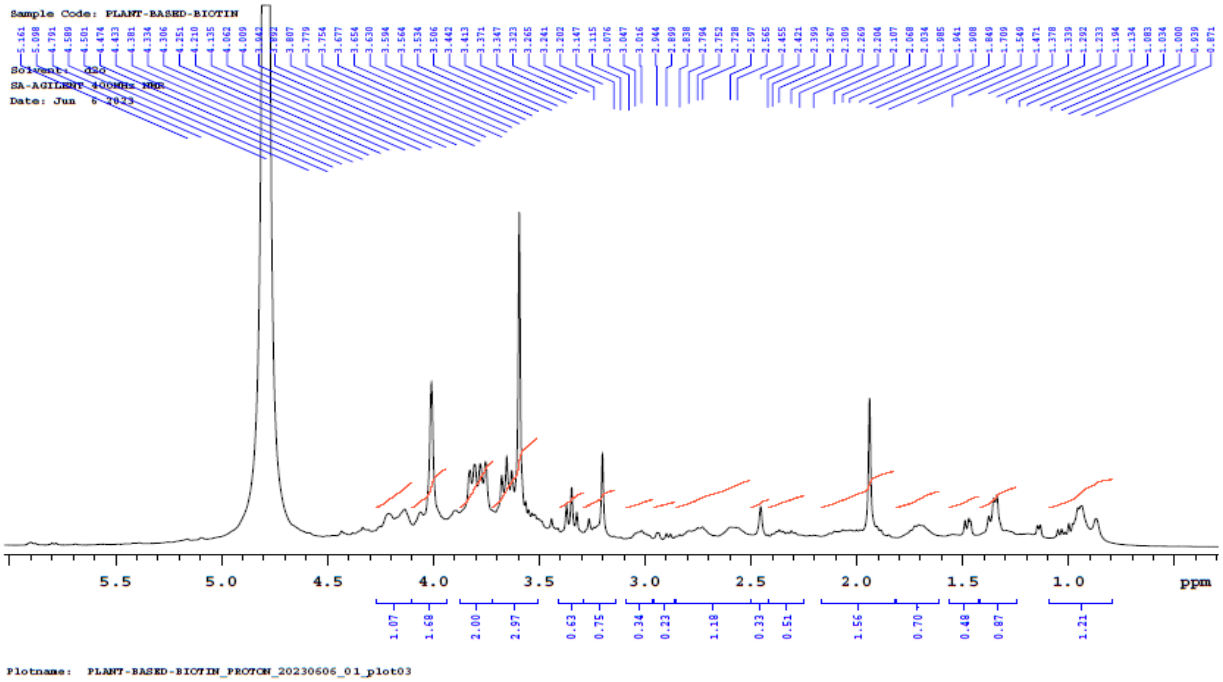

Figure 1: 1H NMR analysis of SesZen-Bio™

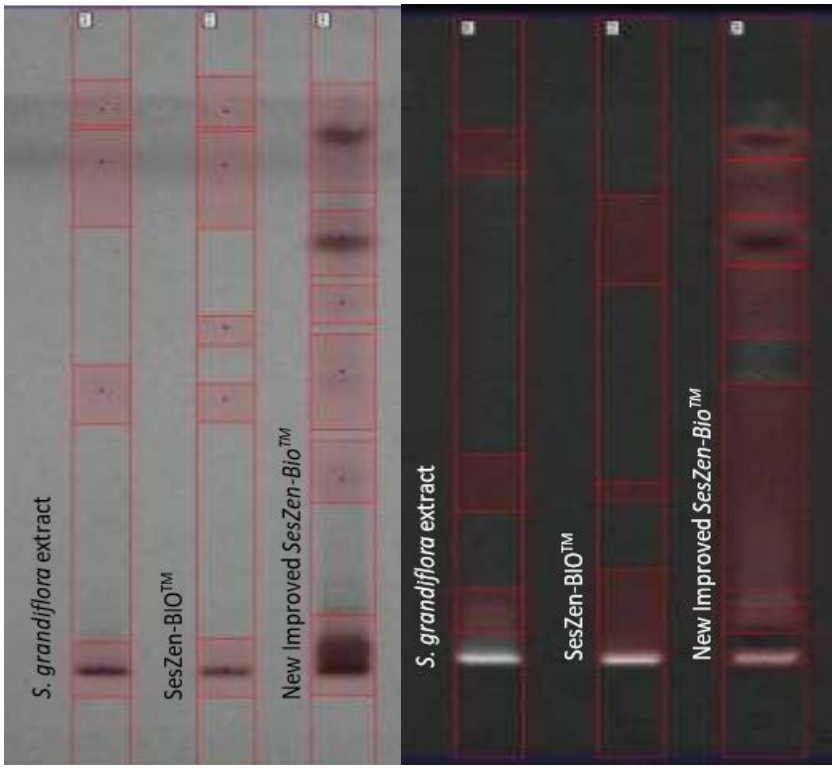

Figure 2: HPTLC plate visualized at 254 nm (left) and 366 nm (right) for *S. grandiflora* leaf extract (reference standard), SesZen-Bio™, and NI- SesZen-Bio™

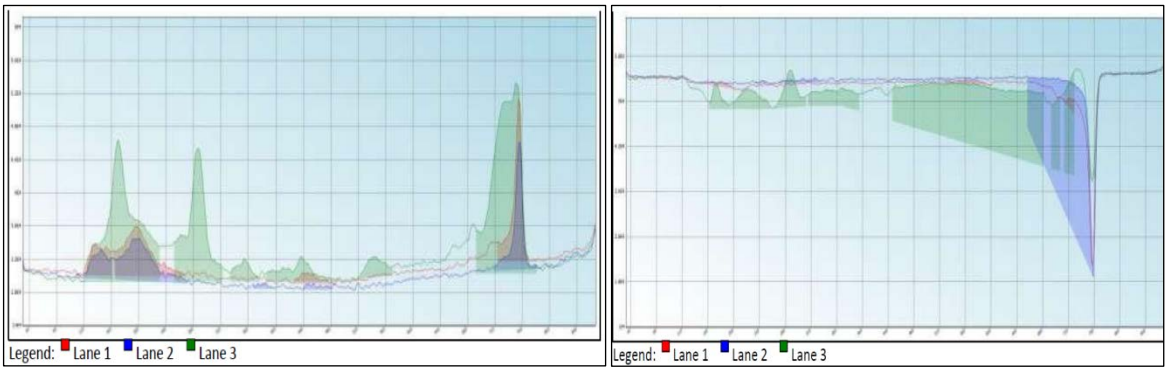

Figure 3: Lane graphs for plates visualized at 254 nm (left) and 366 nm (right) for *S. grandiflora* leaf extract, SesZen-Bio™ and New-improved SesZen-Bio™

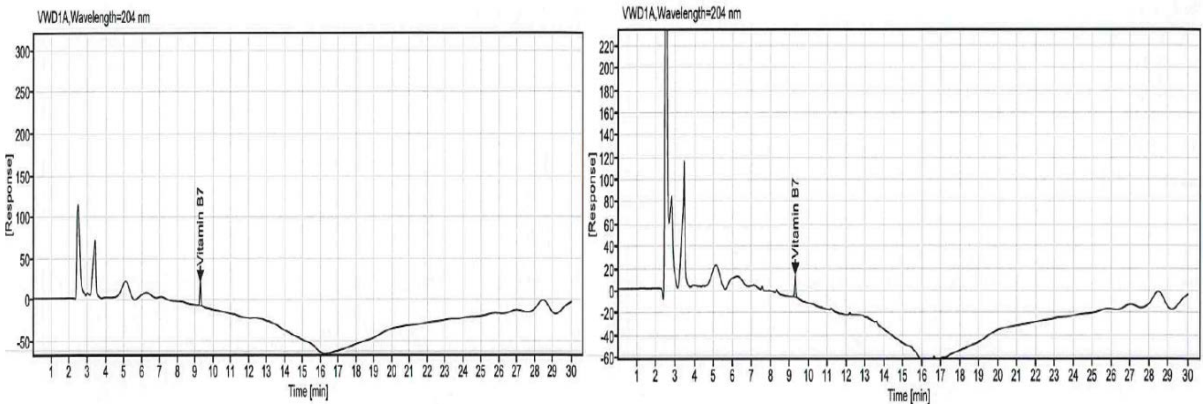

Figure 4: Standard Biotin peak at 9.287 (Left) and Biotin peak at 9.293 in SesZen-Bio™ (Right)

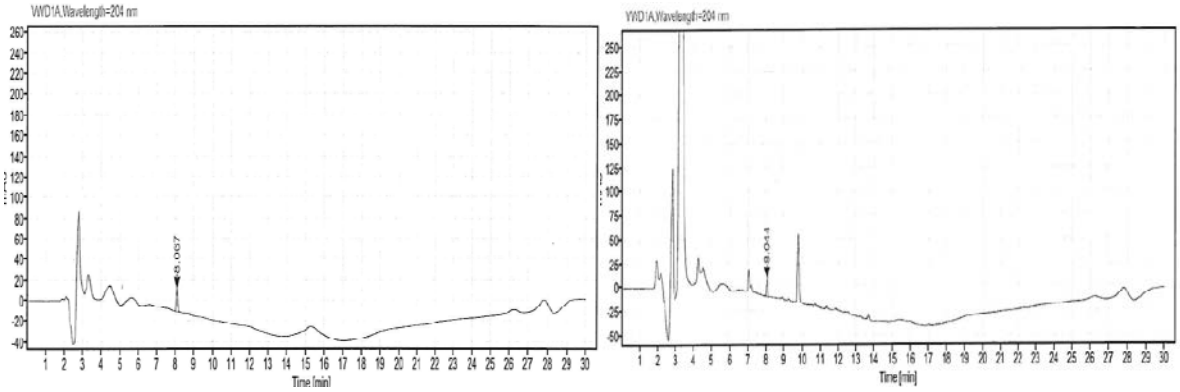

Figure 5: Standard Biotin peak at 8.067 (Left) and Biotin peak at 8.044 in New Improved SesZen-Bio™ (Right)

HPLC analysis

The standard biotin peaked at 9.287 mins, and it was also found in the SesZen-Bio™ at 9.293 mins (Figure 4). Upon calculation, it was found that SesZen-Bio™ showed 0.54% of biotin content. Surprisingly, further decreasing the incubation time of the leaves with 90% ethanol from 6 hours to 4 hours, the biotin content increased to 0.81%. The standard biotin peaked at 8.067 mins, and it was also found in NI-SesZen-Bio™ at 8.044 mins. Thus, the NI-SesZen-Bio™ has 1.5X the amount of biotin (Figure 5) despite almost the same yield of the extract obtained.

Table 1: Phytochemical release study for SesZen-Bio™

Time (mins)	% Release					
	SesZen-Bio™ (mg)				New Improved SesZen-Bio™	
	250	500	800	1000	350	500
60	20.15	18.96	12.48	8.22	15.66	16.57
120	55.89	53.71	45.89	32.56	58.45	45.8
240	84.89	88.93	54.27	48.9	87.9	69.87
360	93.46	97.38	61.29	58.29	96.88	82.45

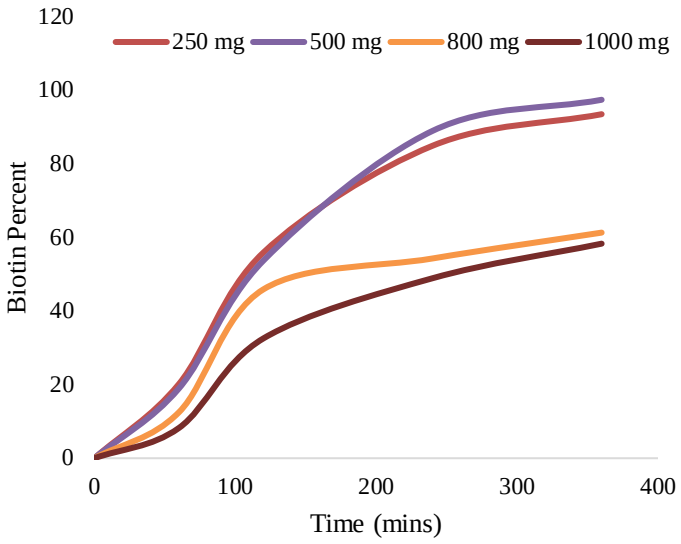

Figure 6: Phytochemical release study

Table 2: Similarity Factor f_2 between different dosages of SesZen-Bio™

	Similarity Factor
SesZen-Bio™ (250 and 500 mg)	74
SesZen-Bio™ (250 and 800 mg)	32
SesZen-Bio™ (250 and 1000 mg)	27
SesZen-Bio™ (500 and 800 mg)	30
SesZen-Bio™ (500 and 1000 mg)	26
SesZen-Bio™ (800 and 1000 mg)	56

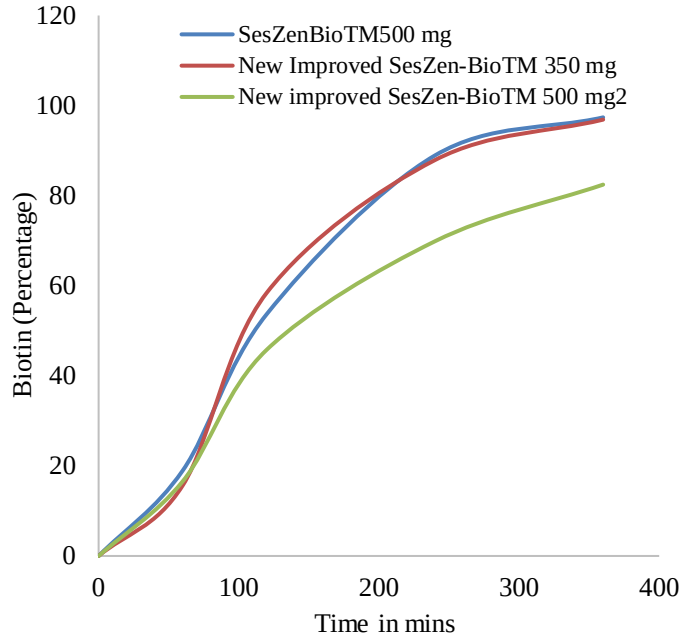

Figure 7: Comparison of Dissolution Profiles for Old and New-Improved SesZen-Bio™

Table 3: Similarity Factor f_2 for between different dosages of OLD and NI-SesZen-Bio™

	Similarity Factor
OLD 250 mg and NEW 350 mg	71
OLD 250 mg and NEW 500 mg	48
OLD 500 mg and NEW 350 mg	72
OLD 500 mg and NEW 500 mg	45

Phytochemical release profile

The results of the dissolution profile were recorded for SesZen-Bio™. As per figure 6, the active ingredient exhibited a 50% release at 120 minutes for both the 250mg and 500mg dosages for SesZen-Bio™ (table 1). Intriguingly, higher dosages at 800 mg and 1000 mg displayed a more prolonged release profile, with the 50% release point occurring after 240 minutes after which the increase was not very significantly high. In contrast, the 250mg and 500mg dosages demonstrated comparable release patterns, whereas the release profile changes significantly above 500 mg of the dosage. Nevertheless, for the NI-SesZen-Bio™ the release pattern was similar to old SesZen-Bio™ till 500 mg/ml that increased with time. The 50% release for improved SesZen-Bio™ was also observed at nearly 120 minutes for both 350 mg and 500 mg doses. Also, as seen in Figure 7, the release study data for NI- SesZen-Bio™ at 350 mg was similar to 500 mg of the old SesZen-Bio™.

The similarity factor (f_2) was calculated for different dosages of SesZen-Bio™, and the dissolution pattern was dependent upon the formulation's concentration. The dosages 250 and 500 mg showed a steady increase in dissolution rate concerning time and bioequivalence with a similarity index of 74 (Table 2). However, after increasing the concentration to 800-1000 mg, the f2 decreased below 50, suggesting a dissimilar dissolution profile. The dissolution profile of NI-SesZen-Bio™ with a higher biotin content was also studied. Both the dosages of 250 and 500 mg of SesZen-Bio™ showed similarity in dissolution with 350 mg of NI-SesZen-Bio™ with higher biotin content with similarity factors 71 and 72, respectively (Table 3). Synthetic biotin was also tested for its dissolution profile. At 100 mg of synthetic biotin showed 42.45% release, which occurred at 60 minutes, and at 120 minutes, complete dissolution was seen for synthetic biotin. SesZen-Bio™ and NI- SesZen-Bio™, on the other hand, showed a sustained release profile and took 360 mins for complete dissolution.

DISCUSSIONS

Sesbania grandiflora contains many phytochemicals in different parts of the plants. The roots contain medicarpin, sativan, and betulinic acid, whereas the flowers contain various amino acids, delphinidin glucosides, and tannins. The seeds contain leucocyanidin, and the plant contains alkaloids, triterpenoids, tannins, saponins, flavonoids, anthocyanins, and other phenolic compounds [13,14]. The ethanol extract of leaves of *S. grandiflora* has also been reported as an anti-tumor medicine with potential comparable to 5-Fluorouracil [15]. In another study, the methanolic extract of *S. grandiflora* was tested for its anti-diabetic potential in type-2 diabetic rats. The 200 mg/kg dosage significantly reduced the increased blood sugar levels and exhibited antihyperlipidemic and reduced insulin resistance [16]. *S. grandiflora* has been reported potential in Ayurveda and Siddha systems as tumor inhibitory, laxative, and anti-helminthic medicine. In a study, it was proven that, by the presence of polymerized phenols and flavonoids, hydroalcoholic extract of *S. grandiflora* leaves exhibited anti-inflammatory potential in acetic acid-induced ulcerative colitis in mice by suppressing the levels of TNF-α and IL-6 [17]. The extract of *S. grandiflora* has also shown potential in the cosmetic sector with its antioxidant and skin-whitening benefits [18].

With the rich phytochemical profile of *S. grandiflora* and its immense potential to treat various disorders, this extract can be a perfect candidate in the nutraceutical sector. Apart from the previously documented data for SesZen-Bio™, the recent study presents an upgraded standardized *S. grandiflora* extract from Ni-SesZen-Bio™ with improved biotin content. Several standardization techniques further improved the quality of NI-SesZen-Bio™ with an increase from 0.5% to 0.8% biotin content with the same yield. The process thus proved economical as the reaction time was reduced from 6 hours to 4 hours while the yield and quality of the final extract were enhanced. The phytochemical release profile was also studied for these two formulations. The release profile was seen to be affected by the concentrations of the sample. As seen in Figure 6, the phytochemical release decreased with an increase in the dosage of the extract. It was also affected by extraction methods (Figure 7). Thus, a thorough extraction strategy is warranted for efficient extraction of the intended phytochemical under study, and determining the dosage is equally crucial. The current study documents, for the first time, the extraction of a biotin-rich blend from Sesbania grandiflora leaves that can be used for hair and skin health. The NI-SesZen-Bio™ extract showed complete dissolution at 360 mins at 350mg and bioequivalence with 250mg and 500mg of SesZen-Bio™. Also, SesZen-Bio™ and NI- SesZen-Bio™ showed a sustained release profile compared to the synthetic counterpart. Besides being natural, NI-SesZen-Bio™ can be taken less frequently due to this ability of sustained release compared to synthetic biotin supplements, and less dosage is required due to its improved dissolution at a lower dosage and higher biotin content. Even though data suggested that biotin deficiency plays a key role in causing brittle nails, hair thinning, and skin disorders, little literature is available on improving skin and hair conditions upon biotin supplementation [19]. Although several biotin supplements are available in the market, this phytochemical-biotin-rich blend holds immense potential in the nutricosmetic market for its ability to alleviate problems associated with hair fall, skin problems, and others. Furthermore, the extract was also tested clinically to prove its hair growth benefits [20], and it was found that consumption of SesZen-Bio™ containing capsules (250 mg twice daily) improved hair density, hair thickness, and ferritin within 8 weeks in comparison to placebo capsules. The NI- SesZen-Bio™ can be utilized at a lower dosage to impart the same bioactivity through its improved phytochemical content, thereby increasing the sustainability index of the product.

As mentioned earlier, the presence of natural phytocompounds with natural vitamins can either increase the bioavailability of these vitamins or increase sustenance between two subsequence dosages compared to synthetic counterparts. Thus, NI- SesZen-Bio™ is a potential candidate for alternatives to synthetic biotin supplements with reported bioactivity, sustained release profile, economical extraction process, and decreased dosage required to deliver the same amount of biotin.

CONCLUSION

The current study focused on improving the economic aspect of the product SesZen-Bio™ using an energy-efficient process. The natural and biotin-enriched SesZen-Bio™ was modified to give NI-SesZen-Bio™ a similar yield but better economic viability and efficacy dynamics. The biotin content was evaluated, and the dissolution pattern of SesZen-Bio™ and NI-SesZen-Bio™ was compared to measure bioequivalence. Compared to SesZen-Bio™, NI- SesZen-Bio™ showed 0.81% biotin content and displayed a similar release profile as SesZen-Bio™, but at a lower dosage of 350mg. It also showed a

sustained release pattern compared to synthetic biotin, which showed an immediate release pattern. Thus, in a landscape where consumers seek alternatives to synthetic supplements, NI-SesZen-Bio™ offers a reliable option for those specifically looking for natural biotin supplementation to maintain skin and hair health. Further studies are warranted to establish a correlation between better bioavailability and understanding the bioactivities of other phyto-ingredients in NI-SesZen-Bio™.

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FINANCIAL ASSISTANCE
Nil

CONFLICT OF INTEREST
The authors declare no conflict of interest

AUTHOR CONTRIBUTION
Mihir Gadani conceptualized the product and the study design. Sneha Badak conducted studies in various laboratories and recorded observations. Ratna Upadhyay interpreted data, conceptualized the study, and drafted the manuscript. All authors read and approved the manuscript for further submission.

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