Characterization of plant-based biotin from *SesZenBio*^{TM} and *in silico* studies to prove its potential in hair care

ABSTRACT:

A standardized extract from *Sesbania agathi* leaves (SESAL) was developed by Zenherb labs Pvt. Ltd., Mumbai, and is one of its kind plant-based biotin supplements. The current work aimed at characterization of SESAL using chromatographic techniques (like HPLC & HPTLC) and DNA authentication. Additionally, an in-silico approach (docking) was also adopted to predict the molecular interactions and probable mechanisms involved in potential role of SESAL in hair care products range. Drug likeliness was assessed on the basis of ADMET properties and Lipinski's Rule of 5. DNA fingerprinting followed by HPTLC confirmed the identification and authentication. The biotin content in SesZenBio was estimated to be 0.5% by validated HPLC method. Mechanistically, biotin along with 2 other cofactors in *SesZenBio*^{TM} is predicted to stimulate the hair follicle growth and differentiation, thus improving hair health. *SesZenBio*^{TM} might hold 2x potential as against synthetic biotin in hair care applications.

Keywords: cosmeceutical formulation, hair follicle, DNA barcoding, HPTLC profiling,

1.0 Introduction:

India has a well-established, traditional medicinal systems which have been accepted globally. Natural drugs are known to be safer with fewer side effects and this has fuelled a huge demand for herbal medicines, across the world (Li et al., 2011). Additionally, we are one of the major contributors in world market of alternative medicines, owing to the rich biodiversity of medicinal plants in India (Marichamy et al., 2014). Last decade has witnessed a surge in plant extracts in formulations for not only pharmaceuticals but also nutraceutical and cosmeceutical applications.

Sesbania, the plant under study, is one such genus (with over 22 species), explored for medicinal, food and cosmetics potentials (Mohiuddin, 2019). Se*sbania*. *grandiflora* and *S. sesban* are the most thoroughly studied amongst all species. *S. grandiflora* is commonly known as agati and belongs to family Caesalpiniaceae Qualitatively, the extracts contain alkaloids, carbohydrates, polyphenols, saponins, tannins, phytosterols and flavonoids. (???) Leaves and flowers of agati show abundance of vitamins (like A, C riboflavin, biotin *etc*.) and minerals (like zinc, selenium *etc.),* while barks and flowers are known to be rich in polyphenols (Zarena et al., 2014, Velusamy et al.2016). There are scare reports of applications of agati leaves extract in cosmetics. Agati contains biotin, which is known for roles in skin health (Dwivedi and Gupta, 2012), and has gained attention for cosmeceutical potential.

Biotin plays roles in protein synthesis, and, helps the body produce elastin and keratin to maintain healthy hair, skin, and nails. By helping the body use important enzymes, biotin also supports a healthy metabolism and nervous system. Biotin cannot be stored in the body, hence, regular supplementation is advised. Foods like nuts, legumes, whole grains, egg yolk etc are natural sources of biotin. and is also produced by the gut microbiota. Due its relatively low cost and easy availability in cosmetic products, biotin supplementation is trending in the looks conscious consumers.

Increased use of herbal medicines has created huge demand for the natural products. To assure the quality of the final product, from handling raw materials with good manufacturing practices to continuous quality control tests using modern and conventional analytical methods, become imperative. These analytical techniques aid in identification, quality evaluation and assessment of relative potencies of the crude drugs. Some of the commonly employed techniques include HPLC, TLC, HPTLC, GC, FTIR, MS etc. (Kumari and Kotecha, 2016). Phytochemical profiling, standardization of herbal formulations, finger print development quantitation is a part of development of plant-based formulations (Jamshidi et al., 2012).

Substitution of the plant raw material or adulteration are the most common challenges posed in the quality assurance of herbal formulations (Pečnikar et, 2014). Thus, newer and reliable methods are needed for authentication of the raw material. Advancements in genomic technologies aid in molecular identification of the plant raw material, using markers. DNA barcoding is one of the most powerful methods to confirm the authentication of plant materials (Li et al., 2015; Sarwat and Yamdagni, 2016).

The efficacy of the drug depends primarily on the interactions occurring at the molecular level. With the advent of computational biological tools, the drug discovery process has got a boost. Molecular docking aims at studying the interactions of ligands that bind to a specific receptor and the identification of its preferred, energetically most favourable, binding pose.

The current study has been undertaken to characterize *SesZenBio*^{TM,} using analytical (HPLC & HPTLC) and genetic methods (DNA barcoding). Also, mechanistic evaluation of the benefits of biotin consumption on hair health has been carried out using computational studies (molecular docking), to gain a deeper understanding of the molecular mechanisms in play.

2.0 MATERIALS AND METHODS:

2.1 SAMPLE:

The sample under study is a plant-based biotin, standardized extract from *S. agati* leaves (SESAL). The product is a proprietary product of the Zen herb lab and finds applications in hair, skin and nails health. The characterization and identification of the extract components were carried out with SESAL

2.2 BIOTIN QUANTITATION:

2.2.1 HPLC

Biotin content in *SesZenBio*^{TM} was quantified using HPLC (Agilent, model-1200). Column was a reverse phase C 18 column (Thermo Accucore, 2.6µm,4.6mm x 150 mm). Mobile phase included solutions A (Water containing 0.025 % TFA:Acetonitrile(90:10v/v)) and B (Acetonitrile) flowing at the rate of 0.7ml /min. Detector-UV detector- 204 nm/210nm

Sample and standards were prepared as per AOAC official method 2016.2 (Official Methods of Analysis (2016) 20th Ed., AOAC INTERNATIONAL, Rockville, MD, Method 2016.02). Standard used was Biotin - Purity ≥99% (Cat. No. B4501; Sigma Chemical Co., St. Louis, MO , USA, or equivalent). Calibration curve was plotted using standard and test samples for quantification of Biotin.

2.2.2 HPTLC

A sensitive HPTLC method was developed for biotin estimation. CAMAG HPTLC used was the with the Scanner 3 for Densitometric Evaluation of Thin-Layer Chromatograms. Two grams of plant powder was added to 20ml of methanol and sonicated at 45°C for 30min. The content was centrifuged at 1500rpm and filtered. Extract so obtained was used as the sample for further analysis. Sample loaded per track was 4µl and developed in mobile phase containing Toluene: Ethyl acetate: Formic acid (6:3:1 v/v/v). Densitometric scan was carried out at 254nm to quantify biotin.

2.3 DNA AUTHENTICATION:

Small pieces of S. agati leaves were used for DNA authentication. Fish DNA barcoding method, suggested by FDA was adopted for DNA extraction (Pawar etal., 2017). DNA was then amplified using standardized PCR and separated by agarose gel electrophoresis. The desired band was eluted and processed for DNA sequencing. DNA Bold and NCBI BLAST programs were used for identification of the plant material.

2.4 MOLECULAR DOCKING:

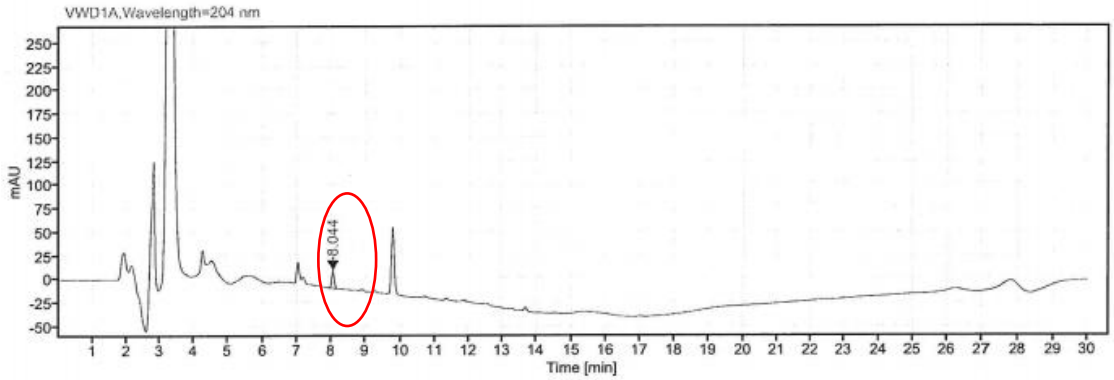
Biotin ($C_{10}H_{16}N_2O_3S$, a significant phytoconstituent from *S. agati* was docked against 3 proteins involved in hair health, based on thorough literature search **(Table 1)**. PubChem (https://pubchem.ncbi.nlm.nih.gov/) was employed to procure the 3-dimensional structures of the biotin in .SDF format and later converted to pdbqt format. Three target proteins, involved in hair health were chosen for the molecular docking, namely- 2HFP, 2P54, and 3LCK (Table 2). Three dimensional structures were downloaded from RCSB (www.rcsb.org). Heteroatoms and water molecules were removed, while Kollman's charges and hydrogen bonds were added to ready the proteins for docking. The protein structures were saved in the pdbqt format. AutoDock Vina 1.5.6. was used to carry out the molecular docking studies in order to understand the interactions of biotin with target proteins that are involved in hair health. To predict the bioavailability of ligand, ADMET profiling was carried out, based on compliance to Lipinski's rule of 5. Bioactivity scores were predicted using the Molinspiration tool (www.molinspiration.com). Biotin was evaluated for its drug-likeliness.

3.0 RESULTS AND DISCUSSION:

Natural, plant-based source of Biotin *SesZenBio*^{TM} standardized to 0.5% Biotin is a uniquely developed, proven all natural, free from toxic residual solvents ingredient derived from authenticated *Sesbania agati* leaves.

3.1 BIOTIN ESTIMATIONS:

HPLC analysis carried out to quantify the biomarkers present in the SesZenBio^{TM} extract, wherein 2 more cofactors were found **(Figure 1)**. These 2 cofactors from SesZenBio^{TM} extract are believed to probably contribute towards its 2X effectivity in hair and skin health.



Signal: VWD1A,Wavelength=204 nm

NAME	Compound Amount Unit	RT [min]	Area	Compound Amount	Peak Tail Factor	PEAK THEORETICAL PLATES USP
Biotin	mcg/kg	8.044	93.19	818.545	0.81	62531.27669
		Sum	93.19			

Figure 1: HPLC chromatogram with biotin peak eluted at 8.044min

Thin-layer chromatography and HPTLC is one of the important separation chromatographic techniques used for detecting adulteration for assessing the quality of the plants through fingerprint profile of the drug (Kumari et al., 2016). If the drug is adulterated there might be the appearance of the other compounds, in turn may increase the no of spots. On the other hand, the exhausted or deteriorated drugs may lose the component, and the number of spots appeared might be less (Li et al., 2011). HPTLC confirmed the quantitation of biotin as 0.5% **(Figure 2)**.

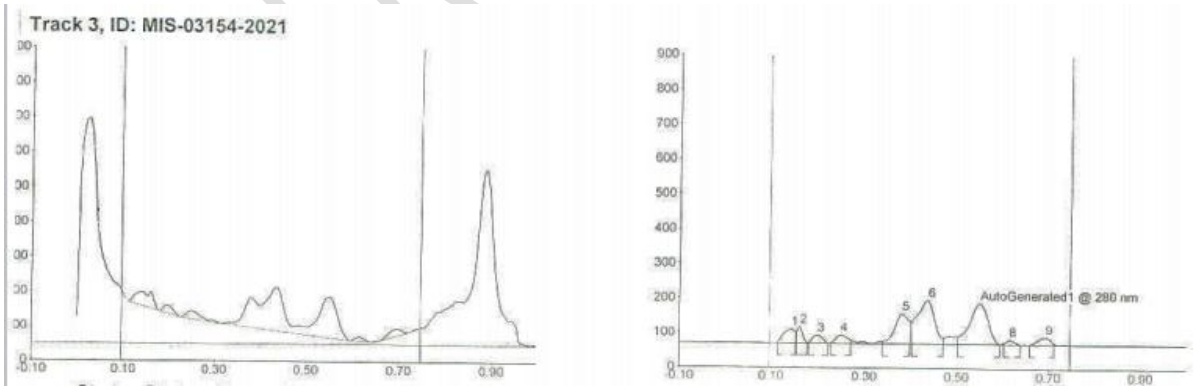
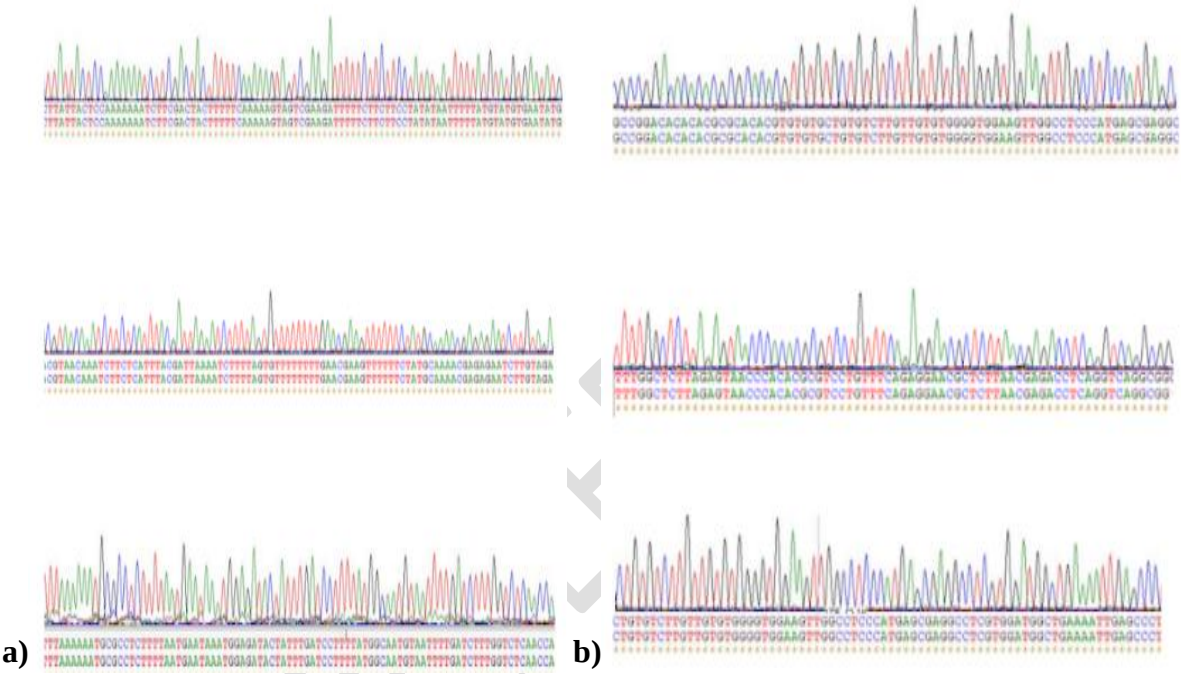


Figure 2: Quantitation of biotin using HPTLC.

3.2 DNA AUTHENTICATION:

Conventionally, taxonomic identifications were carried out based on morphological characteristics, microscopic evaluation, analytical or phytochemistry based methods *etc.* (Techen et al., 2004.) DNA barcoding has emerged as a powerful technique for molecular

identification of an organism. Barcoding involves 3 steps: DNA extraction, PCR amplification and DNA sequencing followed by interpretation. The most common DNA barcodes used in plant identification are fragments from the genes like RuBisCo large subunit (rbcL) and maturase K (matK) (De Boer et al., 2015; Böhme et al., 2019.). In the current study, barcoding was carried out using matK and Internal transcribed spacer 2 (ITS2) markers (Raclariu et al., 2018) **(Figure 3)**. ITS 2 gene of nuclear ribosomal DNA serves as a unique barcode for identification of medicinal plants from a broad range of plant taxa (Ferri et al., 2015, Nithaniyal et al., 2014, Mishra et al., 2016)



a) **b)**

Figure 3: DNA Barcode Markers from *S. agathi* : a) matK and b) ITS2

3.3 MOLECULAR DOCKING:

Biotin (Pubchem ID-171548**)** was docked against 3 target proteins involved in roles in hair health namely-2HFP, 2P54 and 3LCK. The binding energies for the 3 were -6.5, -6.5 and -7.3 kcal/ mol. It complied with all the parameters of Lipinski's rule of 5, indicative of their good bioavailability **(Table 1).**

Ligand	**PARAMETERS OF LIPINSKI'S RULE'S OF FIVE**							
	miLogP	TPSA	natom	MW	nON	nOHNH	nrotb	nvio
Biotin	0.76	78.42	16	244.32	5	3	5	0

Table 1: Evaluation of drug-likeliness of biotin based on the Lipinski's rule of 5.

Peroxisome proliferator-activated receptors (PPARs) are nuclear hormone receptors abundantly expressed in tissues that display high fatty acid metabolism, including human skin and its appendages (Dubrac. and Schmuth, 2011). Three different isoforms of PPARs exist—PPAR-α, PPAR-β/δ and PPAR-γ, which demonstrate diverse functions. PPAR-γ-mediated signalling plays a significant role in the hair follicle (HF) development. PPAR activates the keratinocyte differentiation and accelerates skin barrier recovery. PPAR-γ signalling also promotes the mitochondrial energy metabolism in humans (Kim et al., 2018). Ramot and co-workers have co-workers, have summarized the biological functions of PPAR-γ in skin and hair health, in a review article. Biotin enhances the protein expression levels of PPAR, thus it finds cosmeceutical applications. Continuous stimulation of PPAR-γ by endogenous ligands like biotin, may be required for the maintenance of HF energy metabolism, for intrafollicular PPAR-γ expression and activity and is crucial for the HF physiology (Walth et al., 2018). Biotin deficiency enhances the kinase activity of T CD4 T lymphocytes, which further produces pro-inflammatory molecules. Bioactivity scores **(Table 2)**, displays the effectivity of biotin based on the typical receptors the ligand will encounter in the cell. The score allows efficient identification of active and inactive molecules.

Ligand	BIOACTIVITY SCORES					
	GPCR	ION CHANNEL	KINASE INHIBITOR	NUCLEAR RECEPTOR	PROTEASE INHIBITOR	ENZYME INHIBITOR
Biotin	-0.10	-0.57	-0.62	- 0.63	0.27	0.25

Table 2: Bioactivity scores of biotin.

4. CONCLUSION:

SesZenBio^{TM} has been found to be mechanistically double in hair and skin care potential. This might be attributed to the higher bioactives in the proprietary extract. The extract is derived from green process, hence free from toxic residual solvents. *SesZenBio*^{TM} is proven to have no carbon footprints hence, 100% naturally derived.

Biotin complies with the Lipinski's rule of 5, thus is bioavailable and a potential candidate molecule. It also demonstrates good bioactivity scores. The analytical techniques confirmed the concentration of biotin in the final product. DNA barcoding identified the plant as Sesbania grandiflora, using matK and ITS2 markers.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products.

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