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Evaluation of Antidandruff Activity of Phytochemicals and Molecular Docking Analysis Against Acinetobacter Sp

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Abstract
The human scalp provides a favorable environment for the growth of various microorganisms, particularly bacteria and fungi, which contribute to dandruff. Dandruff is a common scalp condition often associated with disorders like mild seborrheic dermatitis, pityriasis versicolor, and atopic dermatitis. Traditionally, different parts of medicinal plants have been used in hair care and have shown promising results in managing dandruff. This study aims to evaluate the antimicrobial activity of herbal extracts from neem, hibiscus, and onion against of Acinetobacter sps., isolated from dandruff sample. Results revealed that a leading commercial shampoo produced an inhibition zone of 2.1 cm, whereas the herbal extract blend (in equal parts) achieved a larger inhibition zone of 2.5 cm, indicating superior efficacy. Additionally, molecular docking studies were conducted to assess the inhibitory action of bioactive compounds on Acinetobacter lipase, identifying quercetin as a significant inhibitor targeting active site residues ILE36, GLU40, ARG86, and TRP43. These findings support the potential of herbal formulation as effective and safer alternatives for treating mild dandruff.

Keywords: dermatitis, phytochemicals, antimicrobial, MALDI-TOF, anti-dandruff, Acinetobacter sp.

1. Introduction

Human scalp is covered with sebaceous glands which secretes sebum consisting of mixture of fatty acids such as triglycerides. Many factors influences sebum production. Dandruff is a common scalp condition characterized by flaky skin and itching in the head. It is often linked to mild seborrheic dermatitis, pityriasis versicolor, and atopic dermatitis[1]. Human scalp is an ideal environment for various microorganisms, which results in dandruff. Dandruff leads to damaged hair and hair loss if left untreated and the presence of dandruff in the head gives an unpleasant appearance[2].

Acinetobacter sp. species are opportunistic pathogens that are associated with nosocomial infections. They have the ability to form biofilms on human skin[3]. These Acinetobacter is predominantly found at lesion sites[4]. The dandruff causing bacteria such as Acinetobacter produce lipases that degrade fatty acids present in the sebum and thereby a large amount of unsaturated fatty acids are left on the scalp, leading to formation of white scales called dandruff[5]. There are many antibacterial agents that help in managing

dandruff such as zinc pyrithione, chloroxine which are incorporated as active ingredients in the antidandruff hair care products. As these antidandruff hair care products contain synthetic chemcals, toxic side-effects are inevitable. There is a recent trend in improving the shampoo quality by incorporating extracts from well known medicinal plants such as Azadirachta indica, Allium cepa and Hibiscus rosa-sinensis in shapmpoos as an alternative to these synthetic chemicals.

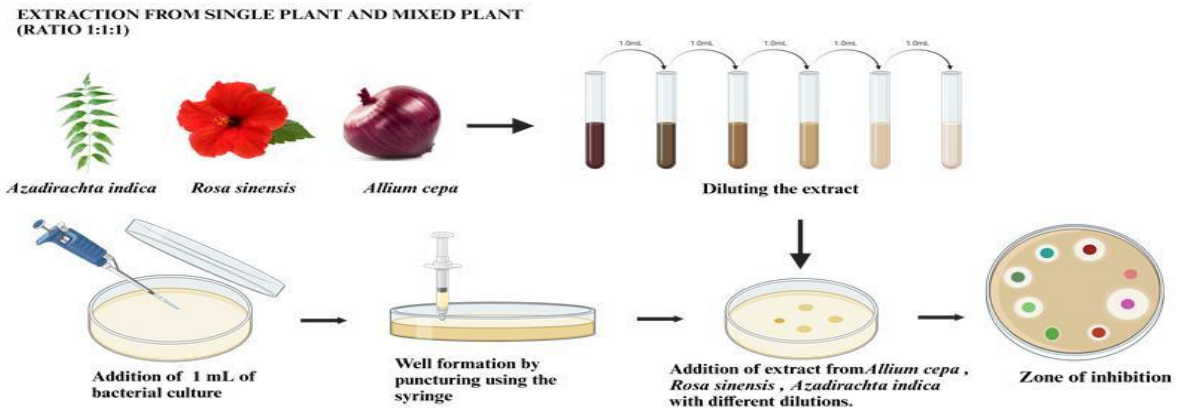
Azadirachta indica, commonly called neem is a native indian tree and it has been reported that several bioactive compounds present in various parts of this plant showed antibacterial properties. . Quercetin is a flavonoid present in A. indica showed antibacterial, anti-inflammatory properties and antioxidant properties[6]. Nimbolinin, Nimbin, Nimbidin, Nimbidol, Sodium Nimbinate are some of the other active components present in A.indica and the most active component is Azadirachtin. Nimbin is an active secondary metabolite, that exhibit strong antimicrobial and antifungal properties[7].

Allium cepa is a commonly used vegetable and is rich in flavonoids, which are polyphenolic compounds known for their wide range of pharmacological activities, including antioxidant, antimicrobial, and anti-inflammatory properties[8]. One of the key flavonoids present in Allium cepa is Quercetin, which has potent antioxidant and anti-inflammatory properties. Quercetin helps to protect cells from damage caused by free radicals and reduces inflammation. Anthocyanin pigments present in them are responsible for characteristic color and possess antioxidant and anti-inflammatory effects. Molecular docking analysis showed that these compounds have high binding affinities for core targets including EGFR, ALB, MMP9, CASP3, and CCL5. Further, components such as kaempferol, luteolin, and other quercetin derivatives show anti-fungal and anti-bacterial activity which marks its efficiency to possess anti-dandruff activity[9].

Hibiscus rosa-sinensis, or hibiscus is a shrub grown throught the world for its beautiful flowers and it is found to contain many bioactive components like flavonoids, anthraquinones, quinines, phenols, terpenoids, saponins, cardiac glycosides, tannins, proteins, amino acids, alkaloids, gum, and mucilage[10]. These components have antioxidant, antimicrobial, anti-inflammatory, and astringent properties that can help to control the growth of dandruff-causing microorganisms on the scalp[18]. Additionally, the alcoholic and aqueous extract of hibiscus leaves has anti-infective, anti-dandruff, and anti-graying properties that aid to darken hair, promote hair growth, and prevention of several skin conditions and allergies[11].

The present study involved isolation and identification of bacteria from dandruff sample collected from a patient from a hair care clinic. Extracts from the dried powder of the three selected plants namely neem leaves, bulb of allium cepa and flower of hibiscus are tested agaisnt the bacterium isolated from dandruff sample and results are compared with commercial shampoos availabe in the market (Figure 1).

Figure 1: Schematic Representation of Antidandruff activity evaluation



2. Materials and Methods

2.1 Isolation of bacterial Culture

The dandruff samples were collected using sterile microfuge tubes via the scraping method, ensuring prior informed consent from all participating individuals. Dandurff samples were obtained from the Skin, Hair & Nail Clinic in RS Puram, Coimbatore. These samples were stored under refrigerated condition until further processing. The samples were then resuspended in nutrient broth and incubated at 37°C for growth and then plated on nutrient agar plate to get individual colonies. One isolate was selected and again grown on nutrient agar plates to get pure isolate and then given for identification at Microbiological Laboratory, RS Puram, Coimbatore. The isolate was identified using MALDI-TOF MS analysis.

2.2 Preparation of plant extract:

The leaves of Azadirachta indica (Neem), flowers of Hibiscus rosa-sinensis (Hibiscus) and bulb of Allium cepa (Onion) were freshly collected, rinsed and shade dried. The shade-dried samples were ground using a home blender. Then 10g of each powder was added to 100ml of methanol and incubated for 24 hours at room temperature with a shaking of 30 rpm. The extract was filtered using Whatman filter paper and dried. The dried extract was collected and stored at 4°C used for further use.

2.3 ADME Analysis

The SMILES format from PubChem was retrieved and inputted into SwissADME web tool and the pharmacokinetic properties of the selected ligands were evaluated.

2.4 Molecular docking analysis

2.4.1 Protein Preparation

The three-dimensional structure of Acinetobacter lipase was chosen from RCBS database in Protein Data Bank (PDB) format. The four letter PDB code for lipase is 4OMP.

2.4.2 Ligand Preparation

The three-dimensional structure of ligands -Nimbin, Anthraquinone and Quercetin were selected for the interaction study and the details of these were derived from PubChem database. The compound ID of Nimbin is 108058 with a molecular formula $C_{30}H_{36}O_9$, similarly the compound ID of Quercetin and Anthraquinone are 5280343 and 6780 with molecular formulas $C_{15}H_{10}O_7$ and $C_{14}H_8O_2$ respectively.

2.4.3 Docking Preparation

The target enzyme and ligands were processed using USFC Chimera 1.18. The additional chains present in the protein were removed and the ligand structures were minimized. Further on, during docking ions, water molecules and charges were added to both the protein and ligands.

2.4.4 Molecular docking process

AutoDock 1.5.7 was used for analyzing the interactions between Acinetobacter lipase and ligands Nimbin, Anthraquinone and Quercetin. The lipase and ligands were taken as output in PDBQT format. The best interactions were selected based on affinity scores, binding energy. The hydrogen bonds between the protein and ligand were also included for further analysis.

2.5 Assessment of Antidandruff activity

Five popularly recommended shampoos for dandruff management were selected for investigation. A known volume of 200µl from each shampoo was utilized for antibacterial analysis via the agar well diffusion method on nutrient agar plates. Then the extract samples were added in different combinations: 200µl of onion extract, 200µl of hibiscus extract, 200µl of neem extract and the final well contained a

200µl mixture of all three samples in four ratios (1:1:1, 2:1:1, 1:2:1, 1:1:2) in different plates containing the same Acinetobacter strain. The plates were incubated at 37C for 24 hour.

3. Results and Discussion

3.1 Isolation and identification of bacteria from dandruff sample

Dandruff sample collected from a patient was cultured in nutrient agar plates resulted in bacterial colonies (figure 2). One colony was selected and further cultured to obtain pure culture isolate and then identified as Acinetobacter from the results of MALDI-TOF MS analysis.

Figure 2: Plates Showing Bacterial Sample (Acinetobactor sp.) Isolated from Dandruff Sample

3.2 ADME Analysis

According to Lipinski rule of five follows the principle that states, no molecule should have more than 10 H-bond acceptors, no more than 5 H-bonds donors, molecular weight should be less than 500 daltons and log P (i.e. octanal-water partition coefficient) should not be more than 5[12]. From the results obtained, Anthraquinone and Quercetin followed Lipinski rule and Nimbin does not follow the rules because of its slightly more molecular weight.

Table 1: ADME Analysis of Bioactive Compounds

Compounds	MW (g/mol)	H-bond acceptors	H-bond donors	Log $P_{o/w}$	Violation
Anthraquinone	208.21	2	0	1.94	0
Nimbin	540.60	9	0	3.68	1
Quercetin	302.24	7	5	1.63	0

3.3 Molecular docking analysis

3.3.1 Protein and Ligand Preparation

The lipase produced by Acinetobacter is responsible for breaking down of lipid layers that protect the scalp, which lead to dandruff[13]. The putative lipase 40PM is chosen as the target protein for docking studies. The natural compounds nimbin, anthraquinone and quercetin from neem, hibiscus and onion respectively were selected as ligand for studying their interactions with this lipase. The target molecule was retrieved from RCBS in PDB format, and the ligands were retrieved from the PubChem database in 3D SDF format which were then converted to PDB format using Open Babel software. The protein and ligands were processed and prepared using Chimera software. The docking preparation includes removal of ions, addition of hydrogen bonds and addition of Gasteiger charges[12].

3.3.3 Ligand Properties

The ligand properties of nimbin, quercetin and anthraquinone were identified using the Lipinski rule of five. The results indicate that all the molecules follow all four rules, except for Nimbin which has slightly high molecular weight. The ligands Anthraquinone, Nimbin has no H donors but Quercetin has 5 H donors, they also have 2, 9 & 7 H acceptors respectively. These compounds interact with the target molecule with the binding energy of -8.4 kcal/mol, -6.6 kcal/mol & -8.6 kcal/mol respectively.

3.3.4 Active site prediction of Acinetobacter lipase

The active site residues were identified using CASTp online tool using the pdb format file of the lipase[14]. The residues found in the active site are listed in the below table 2.

3.3.5 Docking interaction and analysis

The docking studies were carried out using AutoDock Tool 1.5.7. The best interactions were analyzed based on the results of binding affinity and Root Mean Square Deviation (RMSD) values. The molecular interaction between target protein and ligand is illustrated in figure 3.

3.3.6 Interaction between Lipase and Nimbin

The docking interaction studies between Acinetobacter lipase and Nimbin, a major bioactive component in neem, was analyzed in AutoDock Tools as in figure 3(c). From the figure it is evident that the nimbin molecule fits inside the active site of the lipase containing residues ILE36, GLU40, ARG86 and TRP43. This interaction showed an affinity score of –6.6 kcal/mol and RMSD value of 0.791 Å. The hydrogen bonding residues and the bond length are listed in table 3.

3.3.7 Interaction between Lipase and Quercetin

The molecular interaction between bacterial lipase and quercetin, which is the bioactive component present in onion was analyzed in AutoDock Tools as shown in figure 3(a). From the results it is clearly seen that the ligand fits into the active site pocket containing residues LUE77, GLY79 and LEU201. The interaction resulted in a binding affinity score of –8.6 kcal/mol and RMSD value of 0.548 Å. The bond length and hydrogen bonding residues are noted down in table 3.

Acinetobacter lipase also interacts with compounds anthraquinone and nimbin with affinity score of -8.4 and -6.6 kcal/mol respectively. Inhibition of the protein by quercetin is more stable than that of other compounds with respect to affinity scores and location of the binding site. Hence quercetin is an efficient inhibitor for A.lipase.

Insilico results of previous studies are conducted mainly with fungal lipase. The article by Ratish Chandra Mishra et al., shows results obtained from Mflip 1 lipase of Malassezia furfur, their binding energies turned out to be 7.76, -7.43 and -7.34 kcal/mol with 3 different bioactive compounds[15]. On the whole, our results seem to be much better with binding affinities -8.4, -8.2 kcal/mol of quercetin and anthraquinone respectively. Therefore, this strong affinity restricts from binding of bacterial lipase at the active site.

3.3.8 Interaction between Lipase and Anthraquinone

Anthraquinone is a bioactive component highly present in hibiscus, the interaction results between lipase and anthraquinone had an affinity score of –8.4 kcal/mol and RMSD value of 0.00 Å. It is seen that the ligand fits into the active site pocket but doesn't form any hydrogen bond with the lipase. This interaction is represented in figure 3(b).

Table 2: Table Representing Lipinski Rule of Five

S.No	Ligands	Ph value	Mass	Hydrogen bond donor	Hydrogen bond acceptor	Molecular refractivity	Log p
1.	**Quercetin**	7	302	5	7	74.05	2.01090
2.	**Anthraquinone**	7	208	0	2	59.74	2.462
3.	**Nimbin**	7	540	0	9	137.07	3.92239

Table 3: Active Site Residues Present in Acinetobacter Lipase

Protein	Active site residues	Amino acids in active site
Acinetobacter putative lipase	33, 34, 36, 37, 40, 41, 43, 76, 77, 78, 79, 80, 81, 82, 83, 85, 86, 90, 101, 104, 105, 106, 107, 118, 119, 143, 144, 145, 148, 151, 152, 170, 171, 172, 173, 176, 180, 181, 184, 187, 190, 191, 192, 198, 201, 202, 205, 207, 208, 209, 210, 211, 212, 213, 216, 220, 221, 223, 227, 228, 231, 238, 241, 242, 245, 249, 250, 253, 271, 272, 298, 300, 301, 302, 303, 305, 306	PHE, GLN, ILE, LEU, GLU, ARG, TRP, GLY, LEU, ALA, GLY, SER, ARG, ASP, ASN, ARG, HIS, TYR, ASP, GLY, SER, GLY, GLU, VAL, PRO, HIS, SER, LEU, SER, LEU, GLY, ILE, PHE, ALA, TYR, LEU, PRO, LEU, VAL, PHE, LEU, THR, ASN, PRO, PHE, ILE, PRO, PHE, GLN, GLU, LEU, ILE, GLN, ALA, THR, LEU, ASN, TYR, SER, PHE, LEU, ILE, HIS, PRO

Figure 3: Structure of Acinetobacter Putative Lipase

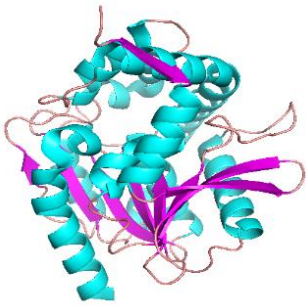
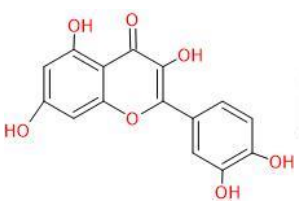
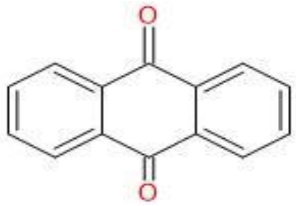


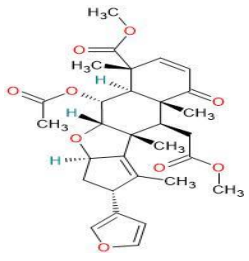
Figure 4: Structure of (a) Quercetin (b) Anthraquinone and (c) Nimbin



(a)

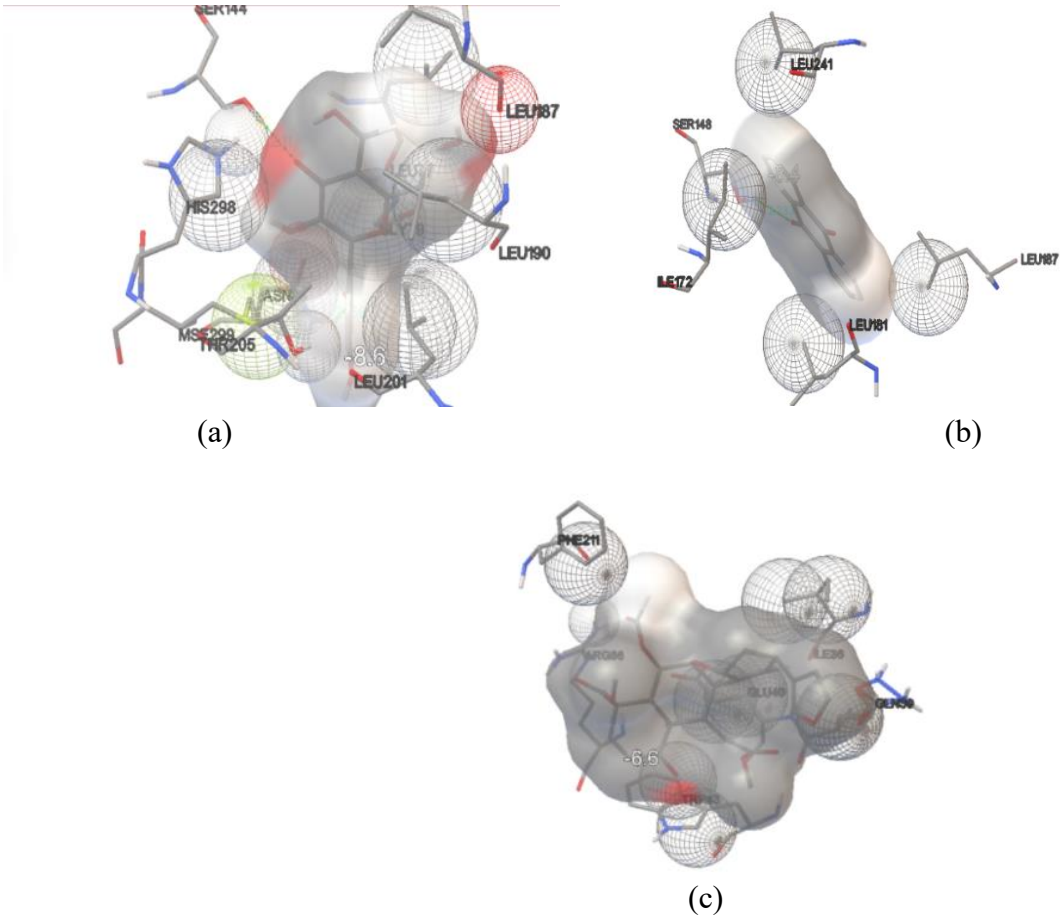


(b)



(c)

Figure 5: Interaction between Acinetobacter lipase and ligands (a) Quercetin (b) Anthraquinone and (c) Nimbin



(a) (b)

(c)

Table 4: Amino Acids Found at the Active Site Upon Interaction Target Protein and Ligands

Ligands	Amino acids interacting
Quercetin	LEU202, THR205, LEU306, ILE212, ARG86, ASN83, PHE198, MSE299
Anthraquinone	ILE172, SER148, LEU181, LEU241, LEU187
Nimbin	PHE211, ILE36, GLU40, ARG88, TRP43, GLN39

Table 5: Molecular Interaction Between Acinetobacter sp. Lipase (target protein) and Ligands

Ligands	No. of H bonds	Amino acids that form H bond	Length of H bond (Å)	Binding energy (kcal/mol)	RMSD value
Quercetin	2	Ser 144 Asn 83	2.11 1.159	-8.6	0.548
Anthraquinone	1	Ser 148	2.233	-8.4	0
Nimbin	0	-	-	-6.6	0.791

3.4 Zone of inhibition

The zone of inhibition between the mixture of extract samples and bacteria resulted to be 2.5 cm.

Figure 6: Plants Showing Zone of Inhibition Against Acinetobacteria sp.

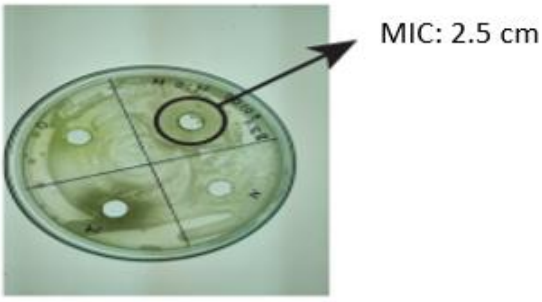


Table 6: Table representing effectiveness and zone of inhibtion against Acinetobacter sp.

Shampoo/ Extract	Zone of inhibition (cm diameter)
Control	No zone of inhibition
Antibiotic (chloramphenicol)	No zone of inhibition
Brand 1	2.1
Brand 2	No zone of inhibition
Brand 3	No zone of inhibition
Brand 4	No zone of inhibition
Brand 5	0.6
Azadirachta indica	No zone of inhibition
Hibiscus rosa-sinensis	No zone of inhibition
Allium cepa	No zone of inhibition
Azadirachta indica + Hibiscus rosa-sinensis + Allium cepa	2.5

3.5. Discussion

The bacterial species Acinetobacter was found to play a major role in causing dandruff in those individuals who poorly maintain their hair. The extracts from neem, hibiscus and onion in combination showed good anti-dandruff effect in terms of controlling the growth of Acinetobacter sp isolated from dandruff sample. Antibacterial evaluation on popular brands of shampoos availabe in the market showed that brand 1 with salycylic acid chemical ingredient showed better results in controlling bacteria than antibiotic chloramphenicol. Currently available ingredients in shampoos that act as anti-dandruff agents are Zinc pyrithione, Salicylic acid, and imidazole derivatives which have the potential to damage hair on long- term use[14].The neem – hibiscus – onion mixture showed good results with a zone of inhibition 2.5 cm diameter and is more advantageous over antibiotic and commercially available shampoos, as the mixture does not possess any undue side effects and they are obtained from natural sources. Further, these materials can be used in combinations on a regular basis to maintain good hair and scalp health.

4. Conclusions

The bacterial species isolated from the dandruff samples identified as Acinetobacter sp. A study was formulated to analyse the effectiveness of selected anti-dandruff commercial shampoos and the extracts obtained from three common plants such as Azadirachta indica (neem), flowers of Hibiscus rosa-sinensis (Hibiscus) and Allium cepa (Onion). Antidandruff shampoo and plant extracts showed a good zone of

inhibition against this bacteria. Combination of 1:1:1 showed a potent inhibition zone rather than using insing indiviual extract which marks its significance to prevent dandruff with affecting the scalp unlike commercial shampoo which has harmful synthetic chemicals. Further, the docking also supported the possible role of bioactive compounds at different actives site in the target lipase of Acinetobacter sp. Therefore, this study proves that the compunds binds to different amino acids at the active site and thereby inhibit the activity of bacterial lipase that causes dandruff.

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