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EVALUATION OF ANTI-DANDRUFF POTENTIAL OF LAWSONE INFUSED IN ALOE–HENNA GEL AGAINST *Candida tropicalis*: TARGETING SCALP VIRULENCE-ASSOCIATED PROTEINS

Sunanditaa Karthikeyan, Aswitha Vijayasekar, Saraswathy Nachimuthu*
Department of Biotechnology, Kumaraguru College of Technology, Coimbatore, Tamil Nadu – 641049, India
*Corresponding author. *Email: saraswathy.n.by@kct.ac.in*

Abstract

Dandruff is a chronic, relapsing scalp disorder driven predominantly by non-albicans *Candida* species, particularly *Candida tropicalis.* It is capable of forming robust biofilm by secreting hydrolytic virulence enzymes. Recent studies show that these organisms are becoming resistant to antifungal agents. Commercial anti-dandruff formulations containing zinc pyrithione, ketoconazole, and salicylic acid are being used to control dandruff and is associated with cytotoxicity and altered hair texture, with diminishing efficacy due to resistance. Hence there is a need for alternative natural formulations that can effectively control dandruff. This study is aimed to formulate, characterize and evaluate invitro antifungal efficacy of Aloe-henna gel incorporated with lawsone (2-hydroxy-1, 4-naphthoquinone) and to validate the pharmacological basis of lawsone activity through in-silico molecular docking against the virulence-associated scalp proteins.

In this study, *C. tropicalis* was identified as the dominant isolate from dandruff samples. The Aloe-henna gel (50 mg/g) showed a zone of inhibition of 16.33 ± 1.52 mm against *C. tropicalis*, which is on a par with Nystatin (17.33 ± 1.52 mm). It showed sustained inhibition persisting till Day 3, where commercial shampoos showed complete re-growth. Drug release followed Fickian diffusion kinetics. Lawsone satisfied standard drug-likeness criteria and demonstrated stable binding to HSA, cutinase, and xylanase, with negative binding affinities indicating spontaneous and thermodynamically favourable interactions. In-silico analyses substantiates the antifungal and pharmacological potential of lawsone, identifying mechanistic interactions with virulence-associated proteins. The all-natural composition of the formulation presents a safer, biocompatible alternative to synthetic anti-dandruff agents.

Key words: Aloe-henna gel; antifungal activity; biofilm; *Candida tropicalis*; dandruff; lawsone; *Lawsonia inermis* L.; molecular docking; scalp mycobiome

INTRODUCTION

Dandruff *(Pityriasis capitis)* is a chronic, relapsing inflammatory condition of the scalp affecting approximately 50% of the global population at some point in their lifetime.[1] While *Malassezia restricta* and *Malassezia globosa* have historically been considered the main cause of dandruff, emerging metagenomic and mycobiome studies have identified a more complex, polymicrobial pathogenesis involving non-albicans *Candida* (NAC) species.[2,3] *Candida tropicalis* has been increasingly recognised as a key contributor to scalp inflammation, flaking, and seborrhoeic dermatitis.[3]

Candida tropicalis is classified as a high-priority fungal pathogen by the World Health Organisation (WHO, 2022) and represents the predominant non-albicans *Candida* species in India, accounting for up to 41% of clinical *Candida* isolates in the Asia-Pacific region.[4,5] Its ability to cause disease in scalp infections is supported by a multifactorial virulence cascade: secretion of hydrolytic enzymes, including secreted aspartyl proteases (SAPs), phospholipases, and esterases; phenotypic switching between yeast and hyphal morphotypes; and importantly strong biofilm formation.[6,7,8] The biofilm matrix functions as a physical barrier that reduces drug penetration and severely limits the efficacy of conventional antifungal therapies.[9]

Dandruff control treatments are majorly comprised of commercial anti-dandruff formulations that rely primarily on zinc pyrithione (ZPT), ketoconazole, and salicylic acid as active ingredients for dandruff control. These active agents, while effective acutely, are associated with cytotoxic potential, alteration of hair texture and follicular health, and increasingly reduced susceptibility arising from mutations in the ergosterol biosynthesis gene *ERG11* (Y132F, S154F) characteristic of *C. tropicalis* azole resistance.[10,11] Furthermore, biofilm-forming *C. tropicalis* demonstrates increased resistance to fluconazole, with 87.5% of biofilm-forming isolates exhibiting elevated MICs compared to planktonic cells.[9] These findings emphasize the need for novel anti-dandruff agents capable of disrupting biofilms and acting through alternative mechanisms.

Lawsonia inermis L. (henna), a member of the Lythraceae family, is a well-documented antimicrobial medicinal plant. Its principal bioactive constituent, lawsone (2-hydroxy-1,4-naphthoquinone), belongs to the 1,4-naphthoquinone class of secondary metabolites.[12,13] Naphthoquinones exert antifungal activity through multiple complementary mechanisms: (i) generation of reactive oxygen species (ROS) via redox cycling, inducing oxidative stress and mitochondrial dysfunction; (ii) disruption of fungal membrane permeability, leading to leakage of intracellular components; and (iii) inhibition of respiratory chain enzymes.[14,15] Critically, these mechanisms are relevant to biofilm inhibition, as oxidative stress disrupts the quorum-sensing pathways that regulate *Candida* biofilm development.

C. tropicalis secretes aspartyl proteases (SAPs) that utilise serum albumin as a primary nitrogen source during infection, and the SAPT family cross-reacts serologically with *C. albicans*-derived SAPs.[16,17] Lawsone binding to HSA may therefore competitively interfere with SAP-mediated albumin hydrolysis.[18] Cutinase, a serine esterase, degrades the cuticular barrier of the skin, and cutinase-like enzymes expressed by pathogenic fungi facilitate host tissue penetration and colonisation; inhibiting this enzyme represents a direct anti-virulence strategy.[19] Xylanase, a glycoside hydrolase, is a component of the extracellular hydrolytic enzyme arsenal secreted by *C. tropicalis* and related species; its activity contributes to degradation of glycoprotein components of the scalp's stratum corneum and biofilm matrix, supporting nutrient acquisition and structural integrity of the biofilm.[20] Thus, the targets (Human Serum Albumin (HSA), Cutinase, and Xylanase) represent mechanistically justified points of intervention in *C. tropicalis* scalp pathogenesis.

The present study therefore aimed to integrate experimental invitro evaluation of an Aloe-henna gel with insilico pharmacological characterisation of lawsone, with a focused investigation of its efficacy and mechanistic basis against *Candida tropicalis* in the context of dandruff pathogenesis.

MATERIAL & METHODS

Clinical sample collection

Ten dandruff samples were collected from individuals between the age group of 18-30 years attending the Skin, Hair, and Nail Clinic, R. S. Puram, Coimbatore, Tamil Nadu, presenting with active scalp flaking with or without pruritus. Written informed consent was obtained from all participants prior to enrolment. Samples were collected by aseptic scraping of the scalp into sterile microfuge tubes and stored at 4^{o}C until processing within 24 hours.

Isolation and identification of Candida tropicalis

Collected samples were inoculated into potato dextrose broth (PDB) and incubated at 25°C for 72 hours. Thereafter, inocula were plated on PDA by the spread plate technique. Individual colonies were sub-cultured on PDA and incubated at 25°C for morphological characterisation. Isolates were submitted for identification to the Microbiological Laboratory, R. S. Puram, Coimbatore, using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS). *Candida tropicalis* isolates were taken forward for antifungal evaluation in this study.

Preparation of Lawsonia inermis L. extract

Lawsonia inermis L. leaves were freshly collected, washed, shade-dried under ambient conditions until complete desiccation. Dried leaves were powdered in a domestic blender. Crude extract was prepared by subjecting 10 g of fine powder to maceration in 100 ml of 50% aqueous ethanol (1:1 v/v) and lawsone was allowed to leach-out for 24 hours. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was evaporated under controlled temperature (45°C) to obtain the dried crude extract.[25,26] The yield was calculated as: Yield (%) = (weight of dried extract / weight of raw sample) × 100. The ethanolic extract was analysed by UV-visible spectrophotometry (UV-1800 Shimadzu spectrophotometer) over the wavelength range 250–650 nm.

Preparation of commercial shampoo samples

Five commercial anti-dandruff shampoos were selected for comparative evaluation: Brand A, B, C, D and E. Working volume of 200 µl of each product was used directly in antifungal assays.

Formulation of Aloe-henna gel

Aloe-vera leaves were soaked upright in water overnight to enable latex drainage and prevent contamination of the gel fraction with aloin. The rind was excised, the inner gel was thoroughly macerated and filtered through linen fabric. The gel was formulated by dissolving 2% xanthan gum (w/v) as the gelling polymer, combining with 5 ml Aloe vera gel diluted 1:1 (v/v) in distilled water, and incorporating *L. inermis* extract as the active ingredient at four concentration levels: 1, 2.5, 5, and 50 mg/g of gel.[28] Xanthan gum was selected as the gelling agent for its pseudoplastic rheological behavior, which enables easy spreading under shear stress during application.

Physicochemical characterization of the gel

Aesthetic and physicochemical properties of the gel including colour, odour, spreadability, washability, visibility, texture, pH, and water content were evaluated using standard methods (Table I). Microscopic texture analysis was performed under a light microscope at 40X magnification. Spreadability was determined by measuring gel diameter expansion under standardised glass slide compression on a 76 × 76 mm slide, and water content was determined gravimetrically after drying at ambient temperature for 24 hours.

Invitro antifungal assay

Candida tropicalis isolates were cultured on PDA plates and standardised to 0.5 McFarland turbidity. Antifungal activity of the henna extract (200 µl), commercial shampoos (200 µl), and Aloe-henna gel (0.5 g) was assessed by the agar well diffusion method. Nystatin served as the positive control and 50% ethanol as the negative control.

Plates were incubated at room temperature and observed on Day 1 and Day 3. The diameter of the zone of inhibition (ZOI, mm) was recorded in triplicate and expressed as mean ± standard deviation (SD).

Drug release kinetics

One gram of Aloe-henna gel was placed on a square glass slide immersed in 50% ethanol as the dissolution medium in a Petri dish. Release of lawsone from the gel matrix was monitored colorimetrically at 300 nm at one-hour intervals over six hours. Cumulative percentage release was plotted against time, and data were fitted to the Peppas-Sahlin model:[29]

$$F = k1t^{m} + k2t^{2m} \quad \ldots(1)$$

where F = fractional drug release, k1 = diffusional (Fickian) constant, k2 = relaxation constant, and m = diffusion exponent. The R/F ratio (k2tm / k1tm) determines the relative contribution of Fickian diffusion versus polymer relaxation: R/F < 1 indicates Fickian diffusion dominance; R/F > 1 indicates Case II transport (anomalous/relaxation-controlled release).

In-silico pharmacokinetic and pharmacodynamic characterisation

The canonical SMILES notation for lawsone (CID 10008, PubChem) was used as input for SwissADME (Swiss Institute of Bioinformatics) and ADMETLab 3.0 to predict physicochemical properties, oral bioavailability, gastrointestinal absorption, blood-brain barrier permeability, CYP enzyme inhibition profiles, and toxicity endpoints. Drug-likeness was assessed against Lipinski's Rule of Five (MW ≤ 500, cLogP ≤ 5, H-bond donors ≤ 5, H-bond acceptors ≤ 10), Ghose filters (160 ≤ MW ≤ 480; −0.4 ≤ cLogP ≤ 5.6), Egan's rules, and Veber's rules (rotatable bonds ≤ 10, topological polar surface area ≤ 140 Å²). Predicted pharmacodynamic properties including antimicrobial and antifungal activity indices, and cytotoxicity parameters, were extracted from ADMETLab 3.0 outputs.

Docking and interaction analysis

Molecular docking was performed using AutoDock Vina (v1.2.0).[30] A grid box encompassing the active site of each target protein was defined with a spacing of 1.00 Å. Docking parameters were saved in a configuration file and docking was executed with exhaustiveness set to 8. The highest-ranked binding pose (lowest binding free energy, kcal/mol) was retained for each protein–ligand complex. Post-docking protein–ligand interaction analysis, including identification of hydrogen bonds, hydrophobic contacts, and π–π stacking interactions, was performed using BIOVIA Discovery Studio (Dassault Systèmes BIOVIA). Two-dimensional interaction diagrams were generated using LigPlot+ (v2.2, EBI).

RESULTS AND DISCUSSION

Identification of Candida tropicalis from dandruff samples

MALDI-TOF MS confirmed that *Candida tropicalis* was predominant among the fungal species cultured from dandruff samples. Colonies on PDA appeared cream-coloured, smooth, and glistening with characteristic budding yeast morphology on microscopic examination. This is consistent with documented scalp mycobiome dysbiosis in dandruff-affected individuals, in whom NAC species proliferate in the altered scalp microenvironment.[3]

Phytochemical extraction and yield

The ethanolic extract of *L. inermis* obtained by the leaching method yielded 10% (w/w) concentrated extract. This yield exceeded the 8.03% previously reported by Rahmoun *et al.*[26] for the same solvent system, attributable to optimised particle size and maceration conditions.

Confirmation of lawsone by UV-visible spectrophotometry

UV-visible spectrophotometric analysis revealed a characteristic sharp absorption maximum at 300 nm (Fig. 1), within the established 280-320 nm range for the naphthoquinone pi-pi* electronic transition.[27] This spectral fingerprint confirmed the presence of lawsone in the extract and validated the redox-active naphthoquinone pharmacophore responsible for its antifungal mechanism.

Physicochemical characterization of Aloe-henna gel

Characterization results are summarized in Table I, Figs. 2, 3. The gel presented a brown colouration comparable to povidone-iodine ointment, mildly acidic pH, fragrant odour, and opaque appearance. Spreadability expanded 2.5-fold under standardised compressive pressure, enabling uniform scalp application. The mildly acidic pH is compatible with the physiological scalp pH range (4.5-5.5), creating an unfavourable environment for *Candida* colonisation which is optimised at neutral-to-alkaline pH. The high water content (71.5%) promotes stratum corneum hydration and enhances lawsone flux across the scalp epidermis. Light microscopy confirmed a finely granulated, homogeneous gel matrix consistent with uniform xanthan gum network formation.

Invitro antifungal activity against Candida tropicalis

Zones of inhibition are presented in Table II and Figure 4. On Day 1, all five commercial shampoos demonstrated inhibition zones ranging from 9.33 +/- 3.05 mm to 35.33 +/- 1.52 mm. Henna extract alone yielded a zone of 8.66 +/- 1.52 mm. The Aloe-henna gel at 50 mg/g produced a ZOI of 16.33 +/- 1.52 mm, activity comparable to Nystatin (17.33 +/- 1.52 mm).

The critical differentiation was observed on Day 3. All five commercial shampoo showed complete regrowth of *C. tropicalis* over zone of inhibition (no inhibition zones), reflecting the transient, rinse-dependent, concentration-driven nature of their synthetic actives. In contrast, both the Aloe-henna gel (50 mg/g) and henna extract maintained *C. tropicalis* inhibition through Day 3, with activity exceeding the 5 mm ZOI reported by Hassan *et al.*[33] at 250 mg/ml henna extract.

The failure of all five commercial shampoos to maintain *C. tropicalis* inhibition beyond Day 1 is mechanistically explained by several converging factors. Zinc pyrithione and ketoconazole act through ergosterol biosynthesis inhibition; their activity is concentration-dependent and wanes rapidly upon rinsing. Lawsone, operating through mechanistically distinct pathways - naphthoquinone redox cycling generating ROS, respiratory chain interference, and membrane permeabilisation[14,15] - circumvents these resistance mechanisms. Importantly, the Fickian diffusion-controlled release from the xanthan gel matrix provides a sustained sub-cytotoxic lawsone flux at the scalp surface, delivering prolonged pharmacodynamic activity that is fundamentally unachievable by rinse-off shampoo formulations.

Drug release kinetics

Colorimetric monitoring at 300 nm demonstrated slow, sustained, near-linear release of lawsone over the six-hour observation period (Fig. 5). Peppas-Sahlin model fitting yielded R/F ratios < 1 at all-time points for all gel compositions (Fig. 6). The diffusional constant (k1) consistently exceeded the relaxation constant (k2), confirming Fickian diffusion through the aqueous xanthan gel network as the dominant drug transport mechanism. This Fickian profile ensures maintenance of lawsone concentrations above the *C. tropicalis* inhibitory threshold over extended periods, providing the pharmacodynamic basis for the sustained Day 3 antifungal activity.

In-silico pharmacokinetic and pharmacodynamic characterisation

Physicochemical, ADME, drug-likeness, and toxicity parameters from SwissADME and ADMETLab 3.0 are presented in Table III. Lawsone satisfied the four standard drug-likeness filters: Lipinski's Rule of Five, Ghose, Egan, and Veber with a bioavailability score of 0.55. One Muegge rule violation was recorded, attributable to the

low molecular weight of the compound falling below the 200 Da Muegge lower boundary, which is generally considered non-restrictive for topical formulations. High GI absorption was predicted, consistent with the compound's moderate lipophilicity (Consensus LogP 1.66) and low TPSA (60.69 A2).

The low skin permeation coefficient (log Kp = −5.77 cm/s) suggests limited skin penetration and predominantly surface-level activity. The detected PAINS and Brenk alerts are associated with lawsone's quinone scaffold, a characteristic feature of natural naphthoquinones that contributes to its ROS-mediated antifungal activity rather than representing a major safety concern."

Molecular docking

Binding affinities, residue interactions, bond types, and interaction counts for lawsone with all three target proteins are detailed in Table IV. Two-dimensional interaction diagrams (BIOVIA Discovery Studio) are presented in Figure 7. The direct electronic engagements with the catalytic acid residue represents the most mechanistically significant docking finding across all three targets.

CONCLUSION

Candida tropicalis, a potent organism with biofilm-forming capacity and multidrug resistance potential, was isolated from clinical dandruff samples and confirmed as the target organism of this study.

The Aloe-henna gel produced a ZOI against *C. tropicalis* on Day 1, comparable to Nystatin with inhibition sustained through Day 3 while all five commercial anti-dandruff shampoos showed complete regrowth as on Day 3. Peppas-Sahlin modelling confirmed Fickian diffusion (R/F < 1) as the dominant release mechanism, providing the pharmacokinetic basis for sustained anti-dandruff efficacy.

SwissADME characterisation confirmed lawsone as a drug-like molecule without Lipinski/Ghose/Veber/Egan violations, bioavailability score 0.55, high GI absorption, and a log Kp of -5.77 cm/s supporting surface retention.

Molecular docking identified distinct active site interactions with all three virulence-associated target proteins: bidirectional hydrogen bonding with THR A:107 of Cutinase (2 bonds; 2 total interactions); H-bond and hydrophobic contacts with ARG A:117, TYR A:138, LEU A:182/185, and ARG A:186 of HSA (8 total interactions); and three conventional H-bonds with GLU A:85 and TYR A:87 plus Pi-Anion contact with the catalytic GLU A:176 of Xylanase (5 total interactions). Together, these findings constitute a multi-target anti-virulence mechanistic basis for lawsone that complements its direct membrane-disrupting antifungal activity. Biofilm inhibition assays, MIC determination, and clinical validation are recommended to advance this biocompatible, all-natural formulation toward therapeutic application.

The all-natural composition of the Aloe-henna gel avoids the cytotoxic synthetic actives of commercial formulations,[11,37] provides Aloe vera's conditioning and cooling scalp effects alongside its own synergistic antifungal anthraquinone activity, and permits long-term use. The addition of aromatic natural oils such as tea tree or lavender oil in future formulation iterations would enhance fragrance and contribute additional antifungal synergy.

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CONFLICTS OF INTEREST

None declared.

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Table I: Physicochemical and aesthetic characterisation of the Aloe-henna gel

S. no.	Property	Method	Result
1.	Colour	Visual observation	Brown
2.	pH	Litmus paper test	Mildly acidic (compatible with scalp pH 4.5-5.5)
3.	Odour	Olfactory assessment	Fragrant
4.	Visibility	Visual observation	Opaque
5.	Texture	Light microscopy (x100)	Finely granulated, smooth, and sticky
6.	Spreadability	Gel placed at centre of 76 x 76 mm slide; second slide pressed under standardised load	Initial diameter 20 +/- 5 mm; expanded to 49 +/- 3.05 mm (2.5-fold increase)
7.	Washability	Exposure to running water	Non-dispersing (stagnant)
8.	Water content	1.12 g dried at room temperature for 24 h; gravimetric determination	71.5%

Table II: Zone of inhibition (mm) against Candida tropicalis on Day 1 and Day 3

+ : growth present (no inhibition); - : complete inhibition

Test specimen	Day 1 ZOI (mm)	Day 3 ZOI (mm)
BRAND A	35.33 +/- 1.52	+
BRAND B	+	+
BRAND C	30.66 +/- 4.04	+
BRAND D	9.33 +/- 3.05	+
BRAND E	9.66 +/- 1.52	+
Nystatin (positive control)	17.33 +/- 1.52	17.33 +/- 1.52
L. inermis ethanolic extract	8.66 +/- 1.52	8.66 +/- 1.52
50% Ethanol (negative control)	+	+
Aloe-henna gel (1 mg/g)	+	+
Aloe-henna gel (2.5 mg/g)	+	+
Aloe-henna gel (5 mg/g)	+	+
Aloe-henna gel (50 mg/g)	16.33 +/- 1.52	16.33 +/- 1.52
Aloe vera gel (vehicle control)	+	+

Table III: In-silico pharmacokinetic, pharmacodynamic, and cytotoxicity properties of lawsone predicted by SwissADME and ADMETLab 3.0

Property	Value / Prediction	Compliance / Remark
Molecular formula	C10H8O3	---
Molecular weight (Da)	176.17	< 500 (Lipinski)
Heavy atoms	13	---
Rotatable bonds	0	< 10 (Veber)
H-bond acceptors (HBA)	3	< 10 (Lipinski)
H-bond donors (HBD)	3	< 5 (Lipinski)
Molar refractivity (MR)	50.02	40-130 (Ghose)
Skin permeation log Kp (cm/s)	-5.77	Low transdermal flux - favourable for scalp retention
Bioavailability Score	0.55	Acceptable bioavailability
BBB permeant	Yes	CNS-accessible under systemic conditions; limited relevance for topical use
Skin sensitisation	Low risk	Suitable for topical application
Serum albumin (HSA)	0.124	Confirms predicted HSA binding; supports docking relevance

Table IV: Molecular docking of lawsone with virulence-associated scalp proteins — binding interactions from BIOVIA Discovery Studio analysis

Target	Interaction type	Residue	Distance (Å)	Total bonds	Significance
HSA Subdomain IIA	Conventional H-bond	*ARG A:117*	2.034	8	Competitive occupancy of albumin binding pocket; disrupts SAP-mediated albumin hydrolysis by *C. tropicalis*
	Carbon H-bond	*ARG A:117:CD*	3.524		
	Pi-Pi T-shaped	*TYR A:138*	5.526		
	Amide-Pi / Pi-Alkyl	*LEU A:185, ARG A:186, LEU A:182*	3.71–5.39		
Cutinase Catalytic cleft	Conventional H-bond (lawsone donor)	*THR A:107:OG1*	2.137	2	Bidirectional H-bond at substrate-binding cleft; blocks access to Ser-His-Asp catalytic triad; inhibits cuticular barrier degradation
	Conventional H-bond (THR donor)	*THR A:107:HG1*	2.444		
Xylanase Active site cleft	Conventional H-bond (lawsone donor)	*GLU A:85:OE2*	2.596	5	Pi-Anion at catalytic GLU A:176 (Glu-Glu dyad); inhibits biofilm

Target	Interaction type	Residue	Distance (Å)	Total bonds	Significance
	Conventional H-bond (TYR donor)	*TYR A:87:HH*	2.133		extracellular matrix degradation and nutrient acquisition
	H-bond (solvent-mediated)	*GOL A:201:HO2*	2.319		
	Pi-Anion (catalytic)	*GLU A:176:OE2*	3.706		

Figures

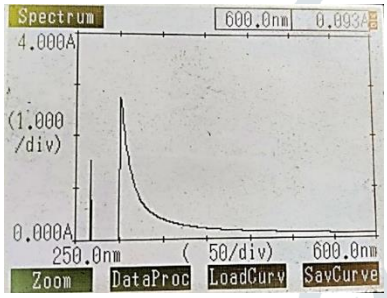


Figure 1: UV-Visible spectrum of Lawsone extract (250 – 650 nm) - Absorption peak

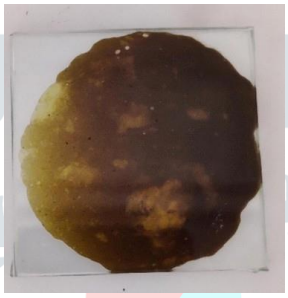


Figure 2: Aesthetic property of Aloe – Henna gel - Spreadability

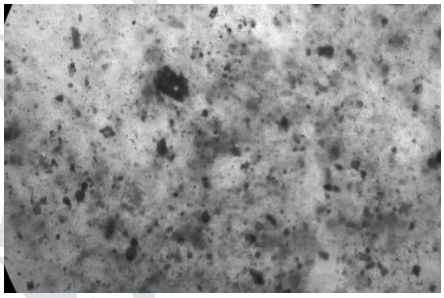


Figure 3 Texture of Aloe – Henna gel under Light microscope (100X)

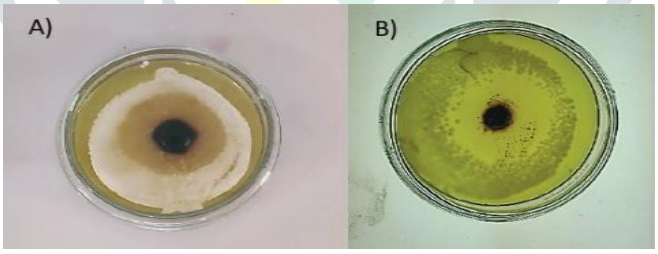


Figure 4 Antifungal activity evaluation - Zone of inhibition for candia tropicalis on Day 3 a) aloe-henna gel , b) lawsone extract ,

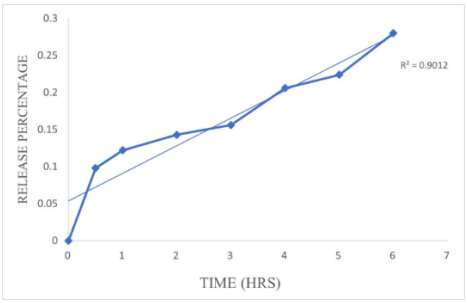


Figure 5: Release of Lawsone from gel by diffusion is represented with respect to time - Release kinetics curve ,

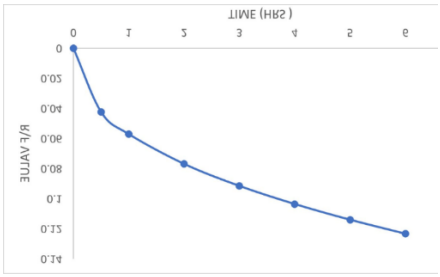
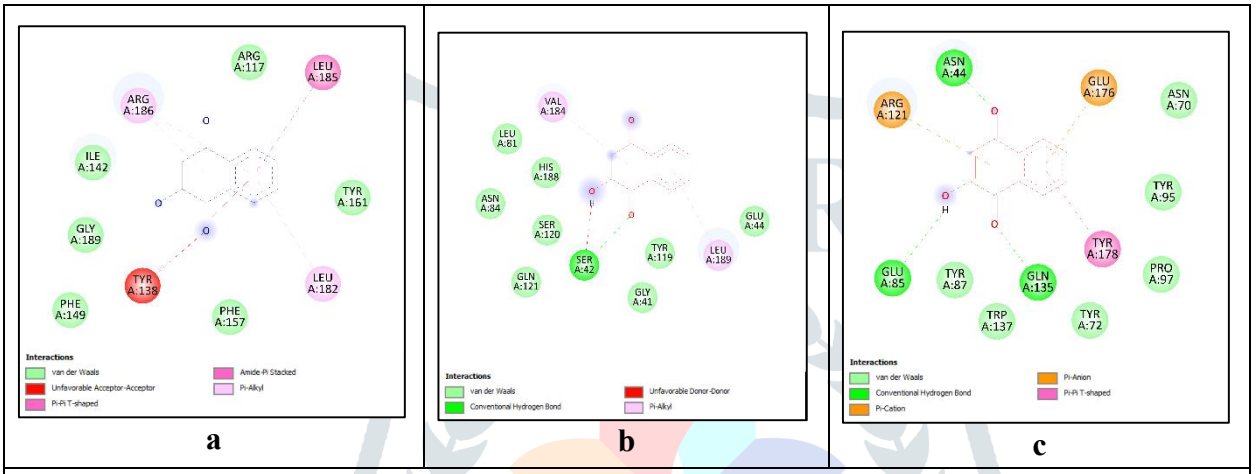


Figure 6: Peppas-Sahlin model of drug release



a **b** **c**

Fig. 7. Molecular docking of lawsone with (a) Human Serum Albumin — H-bond at ARG A:117, hydrophobic contacts at TYR A:138, LEU A:182/185, ARG A:186 (8 interactions); (b) Cutinase — bidirectional H-bonds with THR A:107 (2 interactions); (c) Xylanase — H-bonds at GLU A:85, TYR A:87 and Pi-Anion contact at catalytic GLU A:176 (5 interactions). Interaction diagrams generated using BIOVIA Discovery Studio.