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Design and evaluation of nanosized griseofulvin loaded bio-adhesive layers of acacia catechu (Katha) bio-penetrant for transungual delivery

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Abstract
Topical therapy is highly desirable in treating nail disorders due to its localized effects, which results in minimal adverse systemic events and possibly improved adherence. However, the effectiveness of topical therapies is limited by minimal drug permeability through the nail plate. Infections of foot and hand nails by fungi are a very common condition in millions of people. They account for about half of all nail disorders and are estimated to occur in over 10% of the population. Griseofulvin is an antifungal medication which acts by restraining microtubules and blocking contagious mitosis, consequently a fungistatic. Orally griseofulvin has a long elimination half-life (9-21 hrs), highly variable bioavailability (25to70%), Hepatic demethylation and glucuronidation metabolism and frequent doses(500 to 1000mg) for adult and (11mg) for children. Katha(catechu) is an imperative restorative plant utilized as a part of Ayurveda for such a variety of sicknesses and generally for mother healthcare. Solvent casting technique was used to prepare the bio-adhesive layers. Nine formulations were prepared by using griseofulvin(drug), acacia catechu as bio-penetrant, beetroot as bio-polymer and fructose as film ability. The bio-adhesive layers were physically examined for color and evaluated for thickness, weight uniformity test, folding endurance, Spectral studies, stability studies, in-vitro & ex-vivo studies.

Keywords: Catechu, griseofulvin, nail, beetroot

1. Introduction
Drug delivery to the nail (ungual drug delivery) constitutes a major challenge, with the lack of understanding of both the barrier properties of the nail and formulations to achieve enhanced ungual delivery restricting the efficiency of topical treatments for nail disorders. The currently marketed products Amorolfine and Ciclopirox also suffer from low patient compliance due to the long treatment periods (up to 4-8 months) which are required. However, existing oral formulations typically contain large doses of active ingredients and also require long treatment periods, creating the potential for systemic toxicity especially in the liver. Thus, developing more effective methods for nail drug delivery is an important objective for the pharmaceutical industry. In order to successfully deliver active pharmaceutical ingredients (APIs) across the nail it is necessary to consider the anatomy and physiology of barriers. Using this information one can more effectively utilize drug delivery approaches to maximize the effectiveness of the API – getting the right amount to the right place at the right time. Topical delivery of systemic therapeutics offers benefits but presents a greater technical challenge. Among the benefits, first pass avoidance, convenience and sustained release are most often sited. The nail plate may appear abnormal as result of, a congenital defect, disease of skin with involvement of the nail bed, systematic disease, reduction of blood supply, local trauma, tumors of the nail fold or nail bed, infection of the nail fold, infection of the nail plate.
Onychomycosis Yellow-brown patches near the lateral border of the nail. Beneath the masses of soft horny debris accumulate & the nail plate gradually becomes thickened, broken & irregularly distorted. One or many nails may be affected & there may be associated infection of the skin. Most of the infections are caused by Trichophyton rubrum, *T. inerdigitale*. Onychomycosis, responsible for up to 50% of nail disorders is a very common problem, affecting 3–10% of the population in Europe, prevalence being higher in older people. Occurrence seems to be on the increase due to a growing elderly population, the spread of HIV infection and AIDS, a higher frequency of iatrogenic immune suppression due to the use of immunosuppressant drugs, lifestyle factors such as the wearing of tight-fitting clothing and shoes and the use of communal recreational facilities and health clubs, as well as improved detection and higher public awareness. Most (90–95%) of the infections are caused by dermatophytes, the rest being caused by yeasts and moulds.

Toenails are affected more than fingernails. Toenail onychomycosis are also more recalcitrant and have to be treated for longer durations.

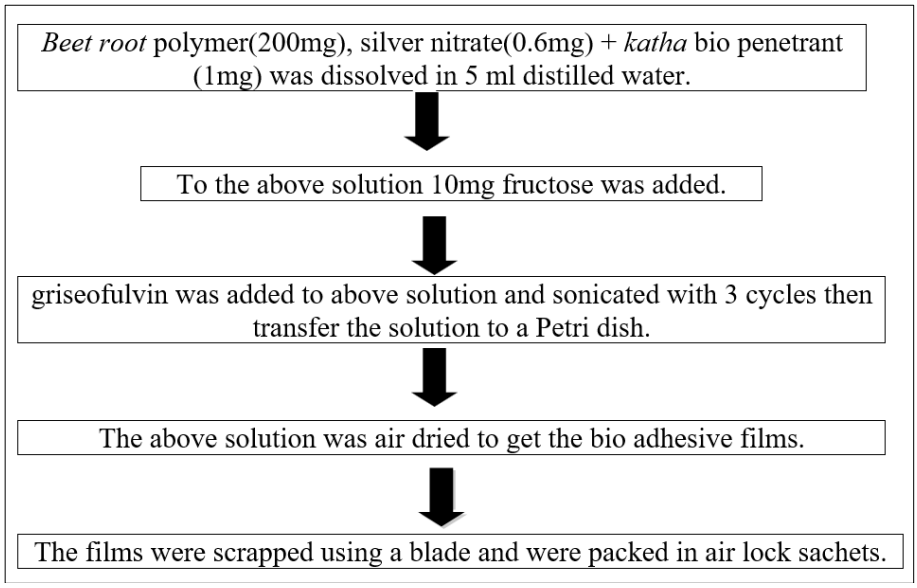
2. Material & Method

2.1. Material- A dark color katha were collected.

2.2. Method

2.2.1. Preparation of bio-adhesive film

Bio-adhesive layers were prepared by solvent casting method. For this the Beet root polymer(200mg), silver nitrate(0.6mg) and the katha bio-penetrant (in various ratios of 0.5%, 1%, 5%, 10%, 15%, 25%, 30%, 35% and 45%) were dissolved in 5ml of distilled water at room temperature in a test tube and then to this polymeric solution 10mg fructose was added. The drug griseofulvin was dissolved in 1ml of methanol in a separate test tube and was then added to the solution containing fructose and the polymer and the bio-penetrant solution and the volume of the mixture was then made up to 10ml using distilled water. This mixture was then transferred into a Petri-dish and the dish was placed on an even surface and was air dried at room temperature and when the film was formed the dried layer was then scrapped out and were packed in air lock sachet. (Flow chart-1)



Flow chart 1: The film was formed the dried layer was then scrapped out and were packed in air lock sachet

2.2.2. Formulations with varying proportions of *katha* bio-penetrant (table-1) -

Table-1: Formulations with varying proportions of *katha* bio-penetrant

Ingredients	KF1	KF2	KF3	KF4	KF5	KF6	KF7	KF8	KF9
Griseofulvin	10mg	10mg	10mg	10mg	10mg	10mg	10mg	10mg	10mg
Bio -Penetrant (*katha)*	1mg (0.5%)	2mg (1%)	10mg (5%)	20mg (10%)	30mg (15%)	50mg (25%)	60mg (30%)	70mg (35%)	90 mg (45%)
Bio-Polymer (*Beet root)*	200mg	200mg	200mg	200mg	200mg	200mg	200mg	200mg	200mg
Silver nitrate	0.6mg	0.6mg	0.6mg	0.6mg	0.6mg	0.6mg	0.6mg	0.6mg	0.6mg
Fructose	10mg	10mg	10mg	10mg	10mg	10mg	10mg	10mg	10 mg
Distilled Water	10ml	10ml	10ml	10ml	10ml	10ml	10ml	10ml	10ml

3. Evaluation Parameter

The bio-adhesive films were evaluated for physical appearance, thickness, folding endurance, drug content, surface pH, nail adhesivity, weight uniformity, drug content uniformity and in-vitro release studies.

3.1 Physical Appearance

The films formulated were visually inspected for various factors like color, clarity, flexibility and smoothness in order to ensure the uniformity in physical appearance of the films. (Table-2)

3.2 Thickness

The thickness of the films from every formulation batch was determined using a digital vernier caliper. Thickness was measured in order to see if there is permissible variation in between all the film formulations. (Table-3)

3.3 Folding Endurance

Folding endurance was determined by repeatedly folding the film at the same place till it broke. The number of times the film could be folded at the same place without breaking was recorded which is known as the folding endurance. (Table-4)

3.4 Surface pH

The individual film was placed in a Petri dish and moistened with 0.5 ml of water and kept for 30 sec. The film surface was brought into contact with the electrode of pH meter and equilibrated for 1 min. This is repeated three times for each film and mean was calculated along with standard deviation. (Table-5)

3.5 Drug Content Uniformity

1 sq.mm of film was cut and dissolved in methanol and volume was made up to 10 ml. 0.1 ml was withdrawn from this and diluted to 10 ml. Absorbance was measured at λ-max of the drug i.e., 237nm by using UV Spectroscopy. From absorbance and dilution factor drug content can be calculated. This was repeated for all the formulations. From the drug content % drug content was calculated. (Table-6) & (Fig.-1)

3.6 Nail Adhesivity
A patch of area 1×1 cm^2 of each formulation was cut down using sharp blade. It was applied over the human nail until it got disadhered. The time of detachment of patch from the nail was noted down that showed nail adhesivity. (Table-7)

3.7 Weight Uniformity
Weight variation test is done in order to ensure the uniformity in weight of the films. The individual films (3 films) from each formulation were weighed on a digital balance and mean was calculated. (Table-8)

3.8 *In-vitro* drug release study
The *in-vitro* drug release study was carried out by using a novel static Modified M.S fish plate diffusion apparatus having two compartments - upper donor and lower receptor compartments. The formulated bioadhesive films were adhered onto a fish plate and it was fixed to a donor compartment at one end with the help of adhesive and ensure that there is no leakage. This assembly was immersed in the receptor compartment which is double walled containing 10 ml of buffer solution of 7.4 pH. Samples were withdrawn completely at regular intervals 1, 2, 3, 4, 5, 6, 17, 24, 48, 50, 52, 54, 56, 58 till 120 hours and replaced by fresh buffer**.** The experiment was continued for 6 days. The samples were analysed by UV Spectroscopy at λ_{max} 237nm. Concentration of drug in sample was calculated from absorbance, slope and dilution factor. Blank was also performed using methanol. The concentration of drug and % CPR (Cardio- Pulmonary Resuscitation) was calculated from absorbance, slope values and dilution factor. (Fig.-2)

4. Spectral studies
4.1 IR Spectroscopy
The result of IR spectra of bio-penetrant isolated from **Acacia** catechu (Katha) showed the peak 1389 cm-1, 3124 cm-1, 2736 cm-1 and 3163 cm-1 which clearly indicated functional groups S=O, RCH=CH2, RHCO and RCO-OH respectively. (Figure-3)

4.2 SEM
The topology of bio-penetrant isolated from **Acacia catechu** (katha) observed irregular, smooth, pletigranule surface topology at 1,000 magnifications. (Figure-4)

4.3 DSC
The DSC of study of bio-penetrant isolated from Acacia catechu (katha) showed sharp endotherm with melting point and glass transition temperature showed 102˚C. (Figure-5)

4.4 NMR
The NMR spectra of bio-penetrant isolated from Acacia catechu (kattha) revealed that the peaks were found to be 27.021 ppm which showed presence of C-C, 79.063 ppm which showed presence of C-O, 119.05 ppm which showed presence of C=C, 143.41ppm which showed presence of C=C, 155.88ppm which showed presence of C=O preferably. Hence it clearly indicated that bio-penetrant was polymeric in nature. (Figure-6)

4.5 Particle size
In spectral analysis, Particle size results of bio-penetrant isolated from Acacia catechu (katha) showed Z-average (d. nm) 3829 respectively.

5. Results & Discussion
The values of physical appearance, thickness, folding endurance, surface pH, nail adhesivity, drug content uniformity, weight uniformity and in-vitro release studies were calculated and graph was drawn.

Table-2: Physical appearance

S.no	Property	Inference (*kattha)*
1.	Colour	Reddish brown
2.	Taste	Bitter
3.	Odor	Odorless
4.	Solubility	Soluble in water
5.	Colour changing point	75-80 °C
6.	Test for Proteins (Xanthoproteic, Ninhydrin test)	+ve
7.	Test for Carbohydrate (Fehling's test, Molish test)	+ve
8.	Test for Starch (Iodine test)	+ve

Table 3: Thickness

S. no	Formulation	Thickness
1.	FT1	0.02
2.	FT2	0.02
3.	FT3	0.02
4.	FT4	0.03
5.	FT5	0.03
6.	FT6	0.04
7.	FT7	0.03
8.	FT8	0.04
9.	FT9	0.03

Table 4: Folding endurance

S.no	Formulation	Folding endurance
1.	FT1	75
2.	FT2	79
3.	FT3	75
4.	FT4	72
5.	FT5	74
6.	FT6	79
7.	FT7	84
8.	FT8	88
9.	FT9	85

Table 5: Surface pH

S.no	Formulation	Surface Ph
1.	FT1	12.08
2.	FT2	11.17
3.	FT3	11.38
4.	FT4	11.26
5.	FT5	12.34
6.	FT6	12.14
7.	FT7	12.29
8.	FT8	12.55
9.	FT9	12.45

Table 6: Drug content uniformity

S.no	Formulation	Drug content uniformity
1.	FT1	80.99
2.	FT2	88.68
3.	FT3	63.80
4.	FT4	56.10
5.	FT5	84.16
6.	FT6	64.25
7.	FT7	108.14
8.	FT8	95.02
9.	FT9	96.38

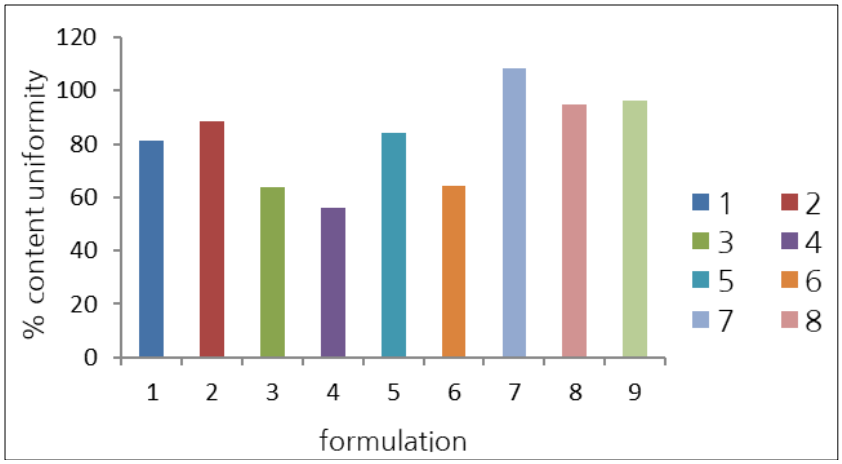


Fig 1: Drug content uniformity of acacia catechu bio-adhesive film

Table 7: Nail adhesivity

S. No	Formulation	Nail adhesivity
1.	FT1	2.52
2.	FT2	2.37
3.	FT3	2.30
4.	FT4	2.42
5.	FT5	3.10
6.	FT6	3.02
7.	FT7	2.54
8.	FT8	2.55
9.	FT9	3.15

Table 8: Weight uniformity

S.no	Formulation	Weight uniformity
1.	FT1	21.43
2.	FT2	24.82
3.	FT3	27.13
4.	FT4	31.4
5.	FT5	30.65
6.	FT6	29.63
7.	FT7	30.67
8.	FT8	31.47
9.	FT9	31.29

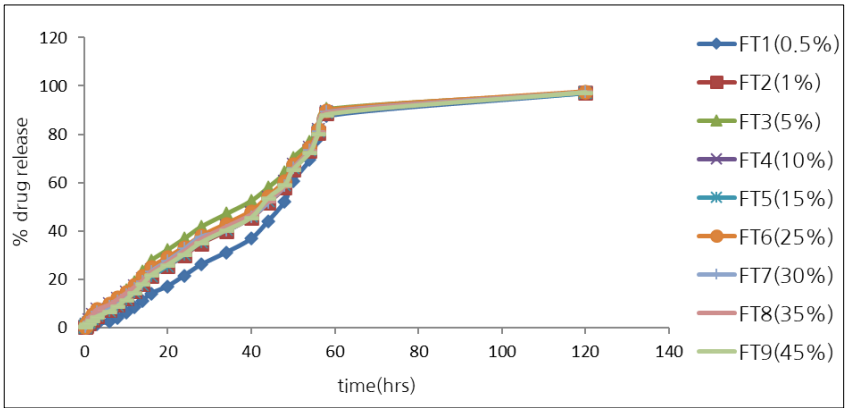


Fig 2: *In-vitro* drug release of acacia catechu (bio-penetrant) by nail clipping

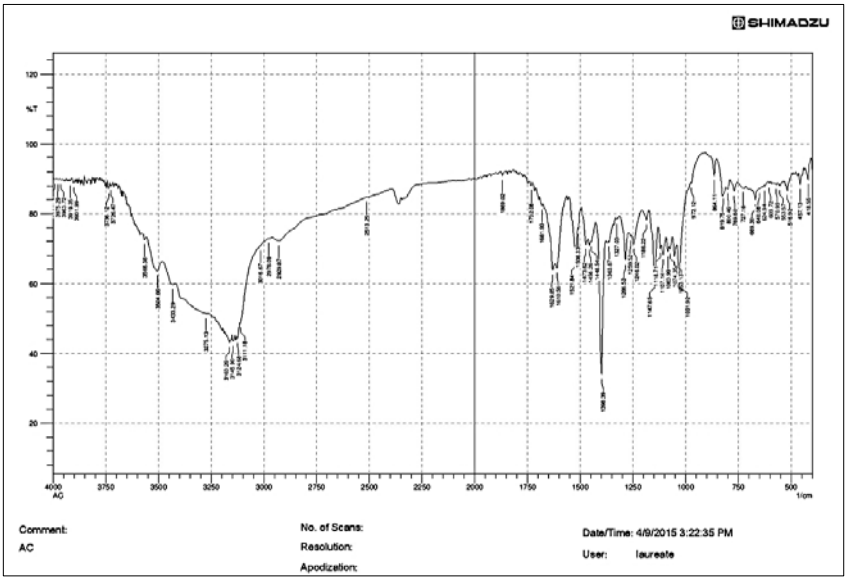


Fig 3: IR report of acacia catechu

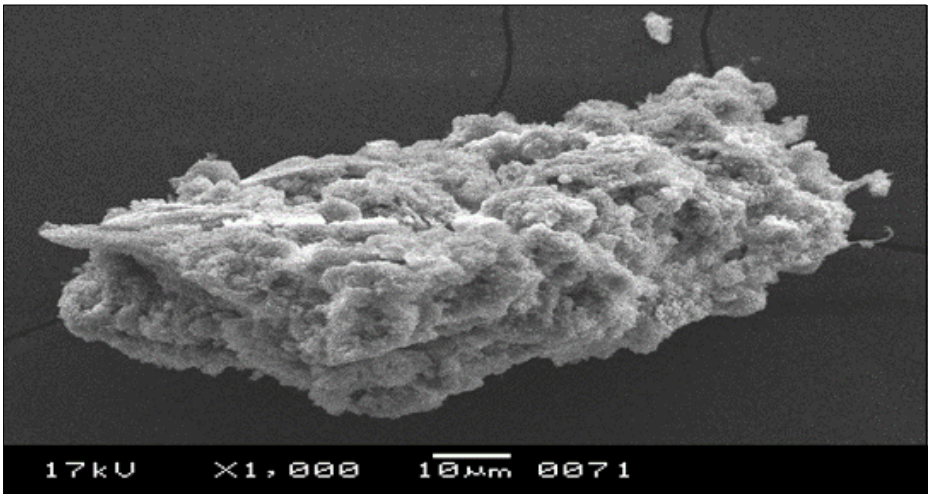


Fig 4: SEM report of acacia catechu

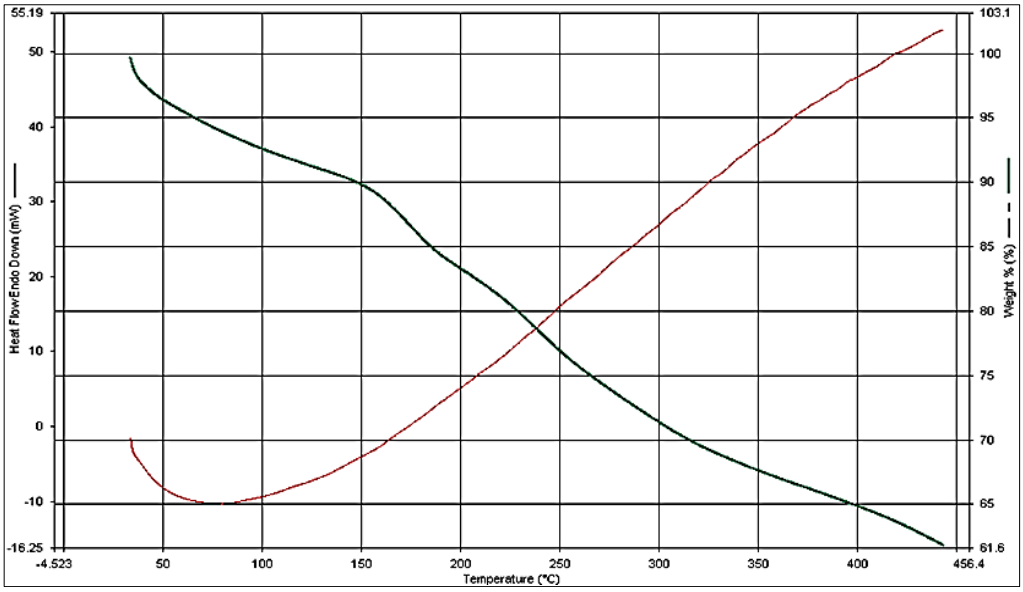


Fig 5: DSC report of acacia catechu

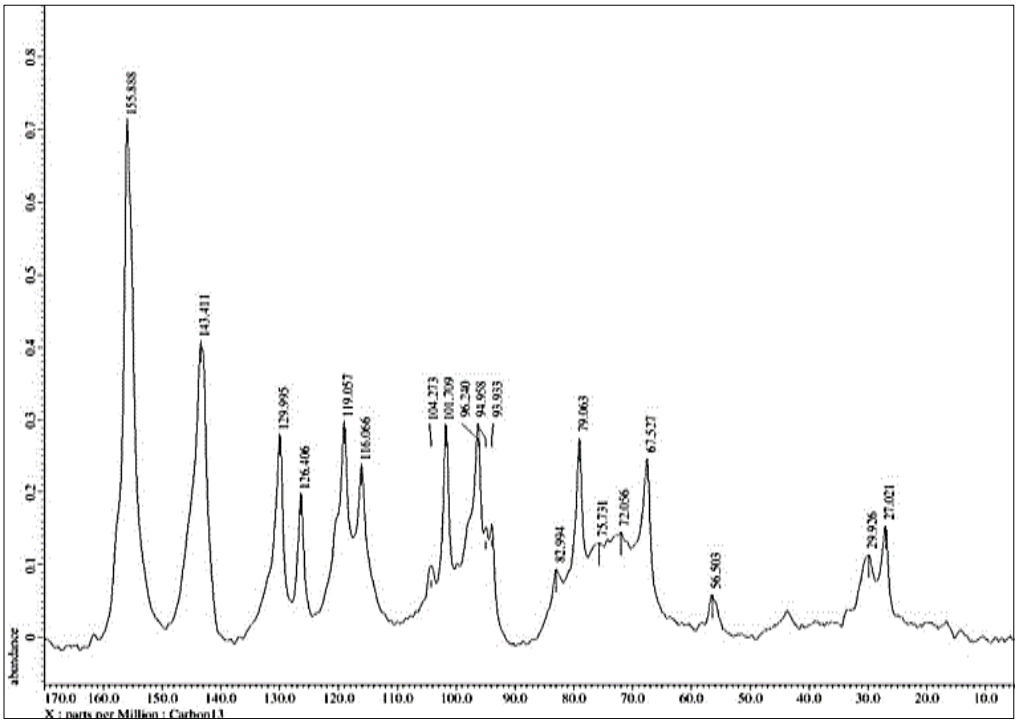


Fig 6: NMR report of acacia catechu

6. Conclusion

Physicochemical properties of Katha bio-penetrant were found to be reddish brown in colour having bitter taste and no odour. It was found to be soluble in water and its color started changing at 75 ℃ and was completely blackened by 80 ℃. When subjected to chemical tests it showed positive reaction for the presence of carbohydrate, proteins and for starch. Thickness of the katha bio-penetrant was (0.02- 0.04), nail adhesivity was (2.30-3.15), weight uniformity was 21.43-31.47, drug content uniformity was 56.10-108.14, suface pH was 11.17-12.55 and folding endurance was 72-88, best formulation was FT8 (35%), R^2 value was 0.9411 shows Peppas Korsmeyer best fit model, mechanism of drug release was Anomalous Transport, T_{50} value was 49.20 & T_{80} value was 78.71. So, Finally I have got one best formulation of griseofulvin loaded bio-adhesive layer of acacia catechu (Katha) bio-penetrant for Transungual delivery.

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