Formulation And Development Of Transdermal Patches Of Amoxicillin And Comparative Effect Of Natural Permeation Enhancers On Ex Vivo Release

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ABSTRACTS:

The motive for this research work was to create and assess matrix type transdermal patches containing Amoxicillin with various polymers and to investigate the effect of extracts of Cumin seeds extracts on the bioavailability of Amoxicillin. The physicochemical similarity of the medication and the polymers were studied by Fourier Transform Infrared (FTIR) Spectroscopy. The outcomes recommended no physicochemical incongruence between the medication and the polymers. The formulated transdermal patches were assessed for weight variation, thickness, folding endurance, moisture loss, moisture absorption, ex-vivo drug release, ex-vivo drug absorption. The diffusion examines were performed by utilizing the Franz Diffusion cell and Everted gut Sac method. The best formulation F25 showed Thickness 0.112±0.004mm, Weight uniformity 0.139±0.004gm, % Moisture uptake 8.154±2.324, % Moisture content 6.121±0.324, % Drug content 82.44±0.231, Folding endurance30±4.11. Formulation F25 exhibits the highest % cumulative drug release 80.34±0.34% in 8hrs and highest %Drug absorbed 4.454±0.21 in 120 min.

Keywords: Amoxicillin, moisture content, FTIR, folding endurance, ex vivo.

INTRODUCTION:

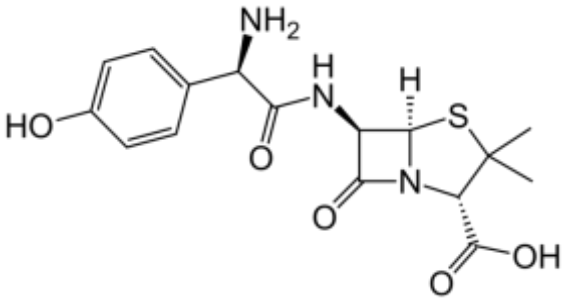

Fig. 1chemical structure of Amoxicillin

(2S,5R,6R)-6-[[(2R)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid

Bioavailability is the rhythm and level to which a therapeutically dynamic substance enters foundational dissemination and gets available at the necessary site of activity. Intravenous medications achieve the greatest bioavailability, while it was seen that oral organization yields a diminished rate because of incomplete medication assimilation and first-pass metabolism[1]

Three fundamental point in particular solvency, disintegration, and intestinal porousness, influencing oral medication assimilation can be assessed utilizing the biopharmaceutics characterization framework (BCS). It arranges the medication into four classes: Class I (high solvency, high penetrability), Class II (low dissolvability, high porousness), Class III (high dissolvability, low porousness) and Class IV (low solvency, low penetrability). A portion of the ordinarily utilized anti-infection agents fall into Class III and Class IV classification as per this framework. One such antibiotic is Amoxicillin which has a low porousness[2]. It is routinely administered as a tablet, oral suspension, or infusion. The medication shows poor oral assimilation because of complete ionization under gastrointestinal pH conditions and shows low lipid solvency also drug has revealed low bioavailability in rat plasma profile[3] It has correspondingly inveterate by the reports obtained from a provincial study in humans[4]

Consistent and long term Concentration of medicament can be achieved by transdermal drug delivery system[5]

In 1982 only US FDA approved Scopolamine transdermal film for motion sickness which is developed by GlaxoSmithKline[6]

USA has approved more than 35 transdermal delivery products for wide variety of pathophysiological condition [7]

TDDS offer numerous benefits over the regular dose structures and oral controlled delivery conveyance frameworks, strikingly shirking of hepatic first-pass digestion, the decline in recurrence of organization, decrease in gastrointestinal results and improves patient consistence[8]

These days, examination into transdermal medication conveyance has extraordinarily expanded in the course of recent years. One of the main impetuses for this development is the expanding number of medications that can be conveyed to the fundamental flow in clinically successful fixation by means of the skin entryway. This has been conceivable in view of the amazing accomplishments of drug technologists who have not just made the transdermal conveyance framework as the best non-oral foundational drug conveyance framework yet in addition made its assembling a profoundly effective advertisement adventure[9]

Transdermal route of administration is the best route for long term and frequent use of drug for maintaining plasma concentration[10]

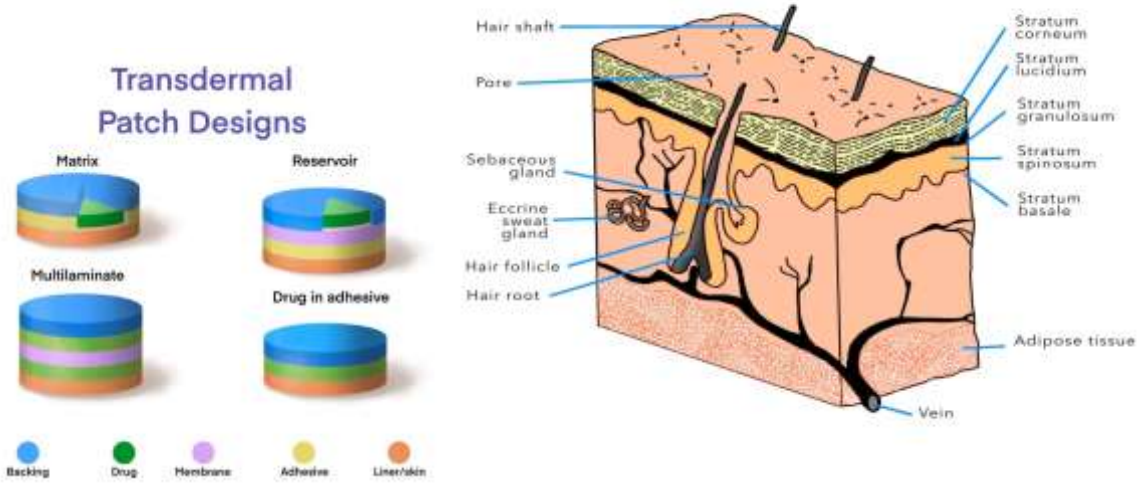

Fig. 2 Transdermal patches design and structure of skin

OBJECTIVE:

Objective of this study is to extract Phytochemical constituent from all the three mentioned herbs and to design and develop Transdermal patch of herbal drug along with modern medicine Amoxicillin using solvent casting method to reduce dose required to obtain same pharmacological effect, also to reduce the toxicity of drug.

MATERIALS AND METHOD:

Materials:

Drug & Chemical

Amoxicillin was obtained as a gift sample from Leben Laboratories Pvt. Ltd. Akola (MH) and other ingredients were obtained from Research lab Mumbai. The entire ingredients obtained were analytical grade.

Plant Material Used

All Plant Materials Black Cumin Seed were obtained from local market impurities andforeign material is inspected then removed and authenticated from botanist.

PLANT PROFILE

a) Black Cumin Seeds:[12]

Figure 3 Black Cumin Seeds

Synonyms Black Cumin Seed Synonyms: black caraway, nutmeg flower, Roman coriander, nigella

Biological Source: It consists of dried seeds of Nigella sativa belonging to family

Ranunculaceae.

Chemical Constituents: Carvone, Alpha Pinene, p-cymene, oleic acid.

Taxonomical Classification:

- Kingdom: Plantae –Vegetal, plants, Planta
- Subkingdom : Viridiplantae – green plants
- Infrakingdom : Streptophyta – land plants
- Superdivisio : Embryophyta
- Division : Tracheophyta – vascular plants, tracheophytes

- Subdivision :Spermatophytina – spermatophytes, seed plants, phanérogames
- Class : Magnoliopsida
- Superorder : Ranunculanae
- Order :Ranunculales
- Family : Ranunculaceae
- Genus : Nigella L.
- Species : Nigella sativa L. – black cumin

Ethno medicinal use:

Antihypertensive, antidiabetic, diuretic, anticancer, analgesic, anthelmintic, antimicrobial, spasmolytic, analgesics and anti-inflammatory, bronchodilator, gastroprotective, hepatoprotective, renal protective, and antioxidant characteristics have all been demonstrated in black cumin seeds. N. sativa seeds are widely used to treat a variety of ailments, including asthma, bronchitis, rheumatism, diarrhoea, and skin diseases. It's also used as a liver tonic, digestive, anti-diarrheal, appetite stimulant, and to help nursing moms produce more milk.

SUCCESSIVE SOLVENT EXTRACTION[13,14]

Black Cumin seeds were extracted by means of successive hot extraction method by using Soxhlet apparatus in order to find out which extract shows the maximum Bio enhancing activity. Extraction was done in following manner

1) Chloroform, 2) Butanol, 3) Methanol, 4) Ethanol 5) Aqueous

Preparation of all extracts by successive extraction method all plant material were air dried at room temperature in order to get consistent weight. The dried samples of all plant material were ground later to coarse powder. Fifty grams of crude powder of bark were taken in Soxhlet apparatus. Successive extraction with different solvents (Chloroform, Butanol, Methanol, Ethanol, and Aqueous) was carried out. Extracts were being filtered using funnel and Whatman No. 1 filter paper. Each filtrate will be concentrated to dryness under reduced pressure at 40°C through evaporator and stored at 4°C for further studies.

PREFORMULAION STUDIES

Melting point: Melting point of drug was found to be 200°C and normal range is 197-204°C

DRUG, EXTRACT AND POLYMER INTARACTION

Fourier-change infrared spectroscopy (FTIR) was utilized to examine the unadulterated medication Amoxicillin, actual blend of Amoxicillin, and HPMC, PG, PEG 400, Glycerine, and ascorbic acid for any medication polymer interaction by KBr pellets technique. All samples were examined at Range: 4000 – 650

STANDARD CURVE OF AMOXICILLIN[15]:

Stock solution of Amoxicillin was prepared by dissolving 100 mg of Amoxicillin in 100 ml standard volumetric flask containing 50 ml of phosphate buffer 7.4 and the solution was then volume was made up to the mark with phosphate buffer 7.4 to obtain a concentration of 1000 µg/ml. Subsequent dilutions of this solution were made with mobile phase to obtain the concentration range of 5- 50 µg/ml. The standard solutions prepared as above were used to obtain calibration curve in order to find the unknown concentration of Amoxicillin, for further study

FORMULATION AND DEVELOPMENT OF TRANSDERMAL PATCHES[16,17]

Transdermal patches were set up by dissolvable solvent casting method. HPMC was weighed precisely and included 3 ml of refined water. The substance in the beaker was blended on magnetic stirrer for 15 min for swelling of the polymer. At that point Propylene glycol was added to the polymer solution. 100mg Amoxicillin was weighed and dissolved in 2 ml of distilled water. The medication arrangement was added to the polymer dispersion and Citric acid was blended altogether with the assistance of magnetic stirrer. At that point After complete mixing solution was allow to stand for 20 minutes to ensure the removal of air bubbles. After that it was poured consistently in Petri dishes and was left for 24hours at room temperature for drying. Subsequent to drying after 24hrs patches were taken out by stripping from the Petri dishes at that point cut into a square component of 2× 2 cm. Patches were stuffed in aluminium foil and put away in a water/air proof holder to keep up their trustworthiness and versatility. The compositions of the various formulations of amoxicillin and extracts are listed in table no. 3

EVALUATION OF TRANSDERMAL DELIVERY PATCHES:

The Physicochemical evaluation of transdermal patches are based on following parameters

Thickness of patch[18]

The thickness of each patch was measured by using screw gauge at five different positions of the patch and the mean value were calculated

Weight uniformity[19]

Patches sizes of 2cm radius (4cm diameter) was cut. The weights of five patches were taken and the weight variation was calculated.

Folding endurance[20]

A patch of 2cm radius (4cm diameter) cut evenly and repeatedly folded at the same place till it brakes. The numbers of times the film were folded at the same place without breaking given the value of the folding endurance

Percentage moisture content[21]

The prepared films were weighed individually and kept in a desiccators containing fuse calcium chloride at room temperature for 24h. After 24h, the films were weighed and determined the percentage moisture content from the mentioned formula.

Percentage moisture uptake[22]

The weighed films were kept in desiccators at room temperature for 24h containing saturated solution of potassium chloride in order to maintain 84% RH. After 24h, the films will be reweighed and determined the percentage moisture uptake from the below mentioned formula.

Drug content[23]

A specified area of patch was dissolved in a phosphate buffer solution. The content was stirred to dissolve the film. The content was transfer to a volumetric flask. The absorbance of the solution were measured and content of drug was determined

BIOENHANCING ACTIVITY MODEL:

Preparation of phosphate buffered saline pH 7.4 [7]

0.19 g of potassium dihydrogen phosphate, 2.38 g of disodium hydrogen orthophosphate, and 8.0 g of NaCl was dissolved in distilled water, and the volume was made up to 1000 ml with distilled water. The pH of the buffer was adjusted to 7.4

A) Ex-vivo Permeation Study[24,25,26]

Goat Skin was obtained from local market and treated properly. Ex vivo permeation studies were performed on Franz diffusion cells with an effective sectional area of 3.14 cm2 and 15 ml of receiver chamber capacity. The treated goat skin was cut into desired size and placed between the receptor and donor compartments of the diffusion cell. The patch was placed over the membrane. The donor compartment was placed on the receptor compartment containing phosphate buffer PH 7.4 maintained at 37+_ 0.5 C and clamp is placed in between donor compartment and receptor compartment for fixing them together entire assembly was kept on magnetic stirrer. The solution in the receiver compartment was uninterruptedly stirrer with magnetic

beads. The amount of the drug infused through membrane was determined by withdrawing particular amount of the sample at programmed time intermission and substituting them with an equivalent volume of phosphate buffer. The absorbance of the samples was taken at wave length 239.00 nm taking phosphate buffer as the blank. Drug absorbance was determined by the standard curve of amoxicillin in phosphate buffer PH (7.4). The amount of drug permeated at each time interval was calculated from the calibration curve. The mean cumulative percentage of the drug permeation through total patch area was plotted against time.

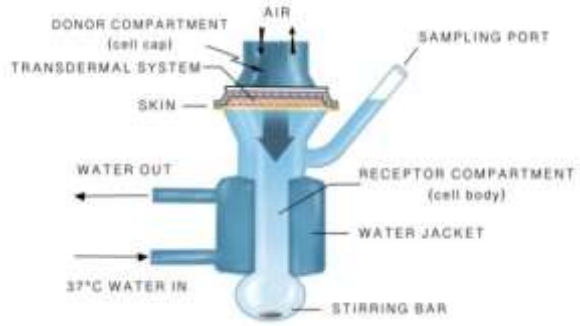

Figure 4 Franz Diffusion Cell

B) Everted Gut Sac Model[27,28]:

Goat small intestine is obtained from slaughtering house from local market. The intestine was transported in buffer solution and cut into 2 pieces each about 15 cm; approximate diameter of intestine was 0.7 cm. One end of the intestine was tied up and everted with the help of glass rod; other end of the intestine was connected to a cannula to form a pouch and added small volume of drug free buffer solution. Continuous supply of oxygen was provided to the tissue in order to keep it alive with the help of aerator and buffer solution; the temperature was maintained at 37± 0.5ºC throughout the entire procedure. After eversion the mucosal side came out and serosal side is present inside. The stratum corneum side of the skin was kept in intimate contact with the release surface of the transdermal patch. At predetermined time the sample from sac was removed and the concentration of drug in serosal fluid is determined with the help of spectrophotometer. Finally % of absorbance was calculated against time.

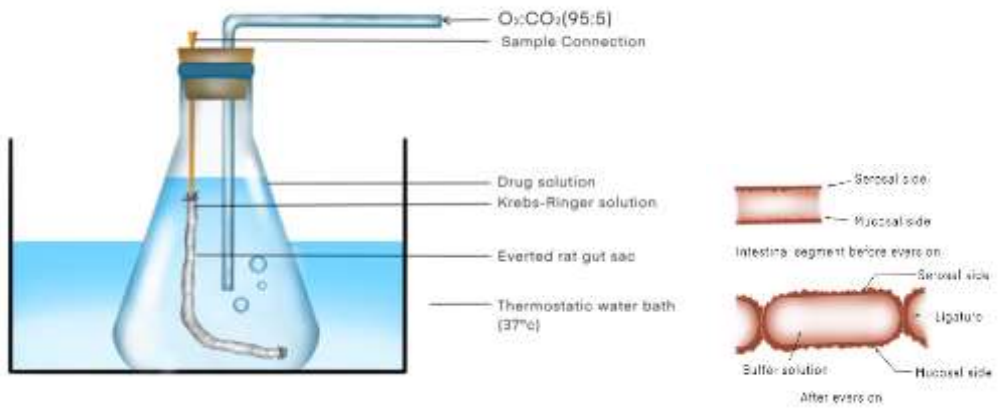

Figure 5 Everted Gut Sac Model

LIST OF TABLES

Table 1 Formulation code table (Amoxicillin+Black Cumin seeds Extracts)

Formulation code	Content
F23	Amoxicillin + Black Cumin Seeds Chloroform Extract
F24	Amoxicillin + Black Cumin Seeds Butanolic Extract
F25	Amoxicillin + Black Cumin Seeds Methanolic Extract
F26	Amoxicillin + Black Cumin Seeds Ethanolic Extract
F27	Amoxicillin + Black Cumin Seeds Aqueous Extract`
F50	Amoxicillin + HPMC+PG+ PEG 400+ Glycerine +Citric Acid

Table- curve of Amoxicillin

S.N	Concentration(µg/ml)	Absorbance
1	5	0.232
2	10	0.443
3	15	0.649
4	20	0.855
5	25	1.069
6	30	1.279

Table 3 Formulation Design for Black Cumin Seed Extract + Amoxicillin

Ingredients	FORMULATION CODE					
	F17	**F23**	**F24**	**F25**	**F26**	**F27**
Amoxicillin	100mg	100mg	100mg	100mg	100mg	100 mg
HPMC	400 mg	400 mg	400 mg	400 mg	400 mg	400 mg
PG	0.4ml	0.4ml	0.4ml	0.4ml	0.4ml	0.4ml
PEG-400	0.4ml	0.4ml	0.4ml	0.4ml	0.4ml	0.4ml

Citric Acid	10mg	10mg	10mg	10mg	10mg	10mg
Water	Up to 5ml	Up to 5ml	Up to 5ml	Up to 5ml	Up to 5ml	Up to 5ml
Chloroform extract	-----	50mg	-----	-----	-----	-----
Butanolic Extract	-----	-----	50mg	-----	-----	-----
Methanolic Extract	-----	-----	-----	50mg	-----	-----
Ethanolic Extract	-----	-----	-----	-----	50mg	-----
Aqueous extract	-----	-----	-----	-----	-----	50mg

Table 4 Evaluation of patches of Black Cumin Seed Extract + Amoxicillin

Parameters	**FORMULATION CODE**					
	F17	**F23**	**F24**	**F25**	**F26**	**F27**
Thickness (mm)	0.322±0.012	0.411±0.021	0.212±0.121	0.112±0.004	0.221±0.145	0.354±0.161
Weight uniformity (gm)	0.121±0.012	0.212±0.010	0.127±0.013	0.139±0.004	0.199±0.121	0.176±0.189
% Moisture uptake	9.143±1.99	8.151±2.145	9.321±1.453	8.154±2.324	9.249±2.343	8.443±2.213
% Moisture content	5.723±0.785	7.675±0.676	6.564±0.967	6.121±0.324	7.098±0.112	6.445±0.675
% Drug content	79.1±0.56	87.21±0.453	78.23±0.67	82.44±0.231	84.67±0.654	85.12±0.564
Folding Endurance	23±2.34	29±4.23	27±3.12	30±4.11	32±3.42	28±4.48

*All data are presented in Average ± SD, n=3

Table 5 %CDR of Black Cumin Seed Extract + Amoxicillin patches

Time in hrs.	**FORMULATION CODE**					
	F17	**F23**	**F24**	**F25**	**F26**	**F27**
0.5	4.15 ±0.54	5.11 ±1.12	5.34 ±0.23	10.45 ±0.12	7.23 ±0.64	4.20 ±0.11

1.0	6.37 ±1.09	7.56 ±1.14	8.44 ±1.23	16.23 ±0.23	12.34 ±0.65	6.67 ±0.11
1.5	8.45 ±1.34	10.53 ±0.67	12.17 ±0.12	23.14 ±1.45	19.16 ±0.98	8.67 ±1.36
2.0	11.57 ±0.78	16.15 ±1.34	22.13 ±1.21	31.45 ±0.45	28.14 ±1.34	11.88 ±0.32
2.5	14.45 ±1.65	20.56 ±0.54	28.43 ±0.34	40.35 ±1.34	36.12 ±0.23	15.23 ±0.21
3.0	15.61 ±0.12	24.15 ±1.13	34.11 ±1.32	48.34 ±1.23	42.12 ±1.56	15.90 ±0.56
4.0	19.25 ±0.41	29.54 ±0.56	38.65 ±1.87	52.34 ±1.42	47.21 ±1.64	20.23 ±0.34
5.0	30.81 ±1.03	36.23 ±1.34	40.17 ±0.26	58.43 ±1.56	53.12 ±0.43	31.56 ±1.32
6.0	39.75 ±1.15	44.12 ±0.67	47.19 ±0.32	67.15 ±1.15	62.17 ±1.54	40.12 ±1.23
8.0	54.45 ±0.81	59.12 ±0.23	60.67 ±0.57	80.34 ±0.34	74.14 ±0.15	55.19 ±1.23

*All data are presented in Average ± SD, n=3

Table 6 %Drug absorbed of Black Cumin Seed Extract + Amoxicillin bulk drug

Time in Min.	FORMULATION CODE					
	F17	**F23**	**F24**	**F25**	**F26**	**F27**
10	0.642±0.24	0.690±0.11	0.750±0.17	0.791±0.14	0.772±0.71	0.654±0.42
20	1.124±0.31	1.184±0.34	1.277±0.23	1.531±0.16	1.478±0.23	1.131±1.14
30	1.412±0.19	1.420±1.12	1.423±0.77	1.844±0.22	1.647±0.19	1.416±1.23
60	1.747±0.52	2.401±1.14	2.754±0.17	3.359±0.36	3.171±0.36	1.753±0.22
90	2.242±1.01	2.778±0.27	3.114±0.96	3.887±0.17	3.662±0.74	2.249±1.03
120	2.704±1.12	2.998±0.96	3.404±0.17	4.454±0.21	4.143±0.39	2.710±0.91

*All data are presented in Average ± SD, n=3s

LIST OF ADDITIONAL FIGURES

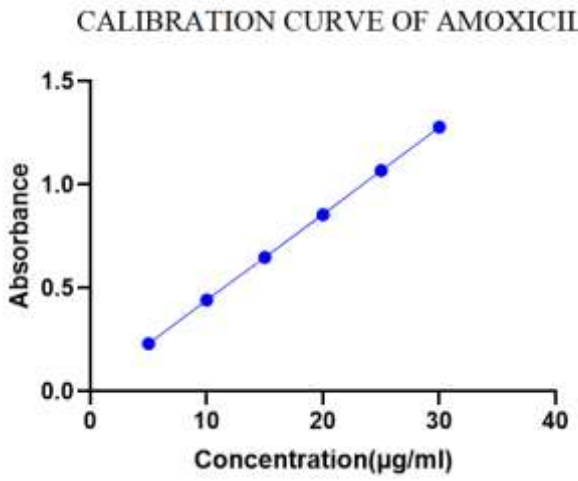

Figure 6 calibration curve of Amoxicillin

F17

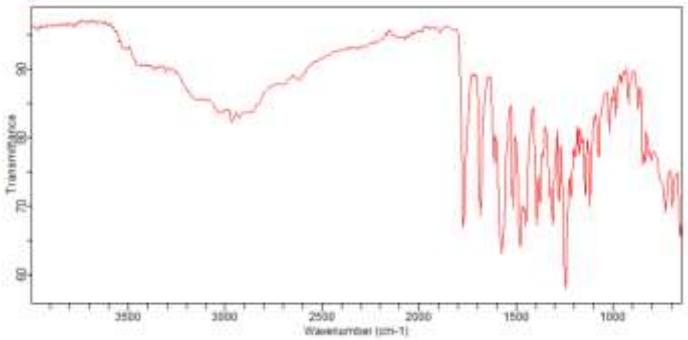

Figure 7 FTIR of Amoxicillin Bulk Drug

F23

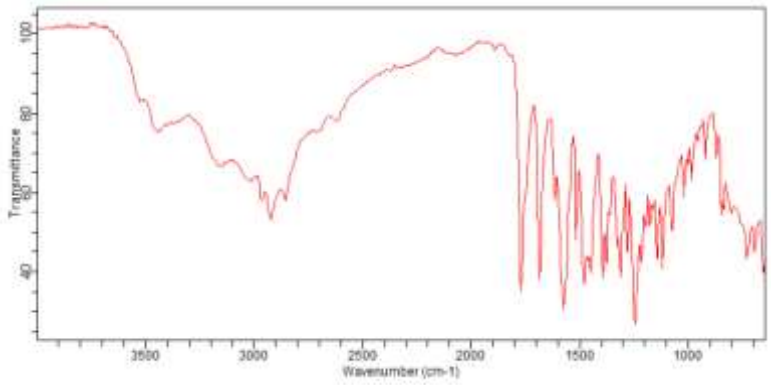

Figure 8 Amoxicillin + Black Cumin Seeds Chloroform Extract

F24

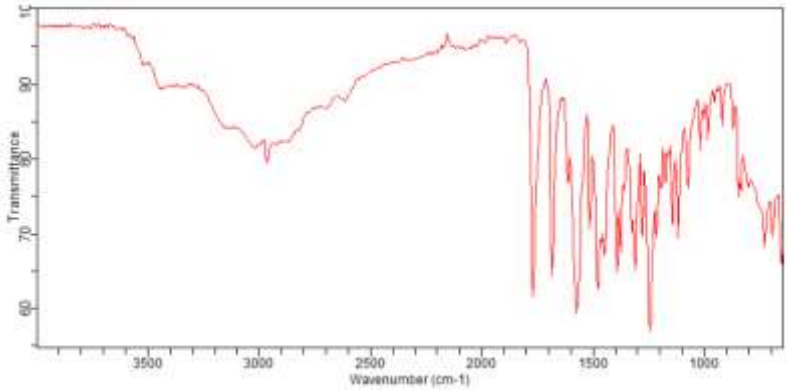

Figure 9 Amoxicillin + Black Cumin Seeds Butanolic Extract

F25

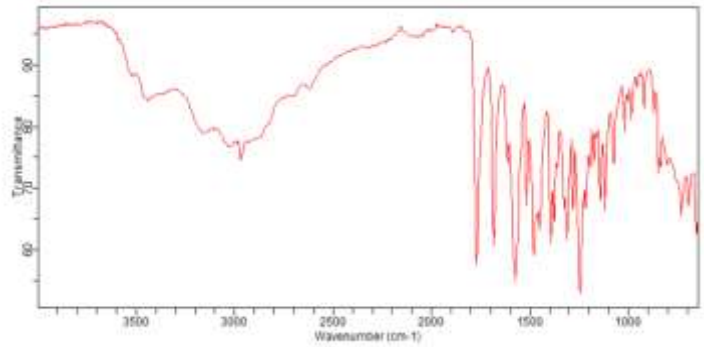

Figure 10 Amoxicillin + Black Cumin Seeds Methanolic Extract

F26

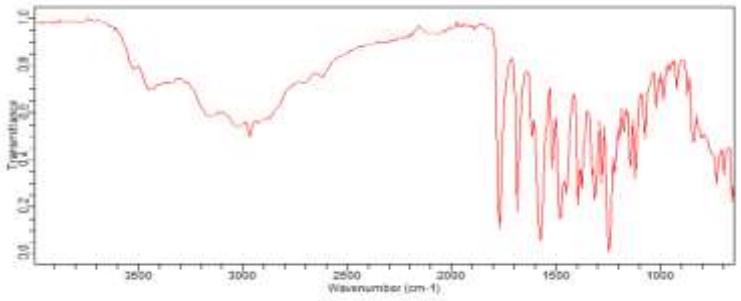

Figure 11 Amoxicillin + Black Cumin Seeds Ethanolic Extract

F27

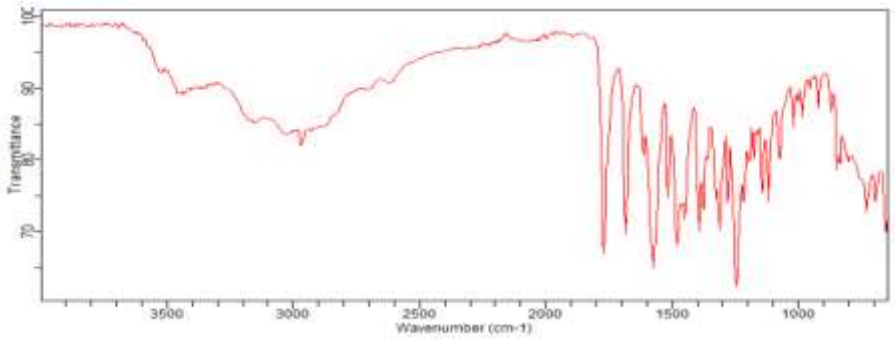

Figure 12 Amoxicillin + Black Cumin Seeds Aqueous Extract

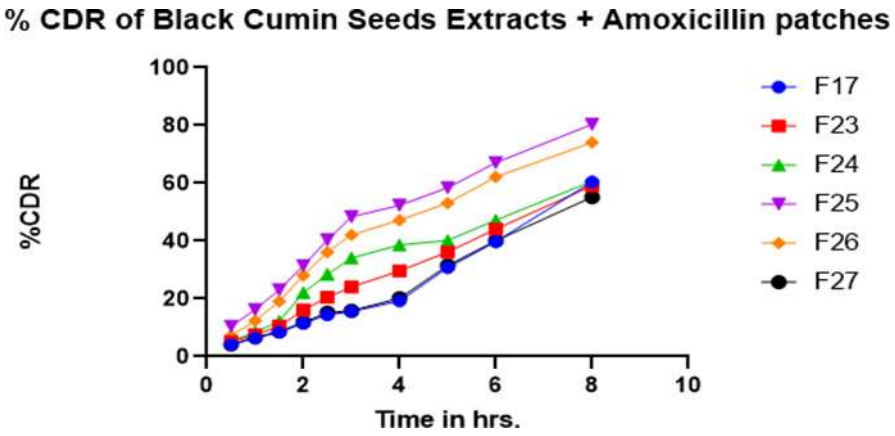

Figure 13 % CDR of Black Cumin Seeds Extract and Amoxicillin

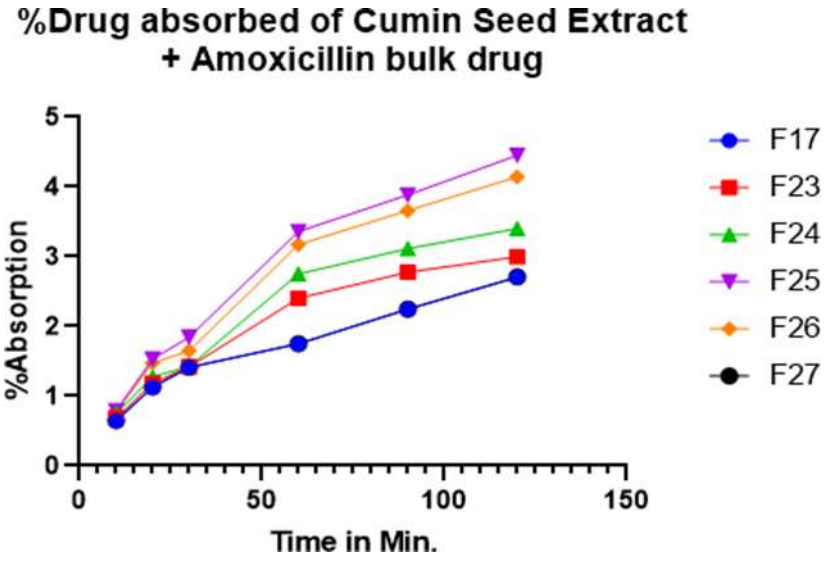

Figure 14 % Drug Absorbed of Black Cumin Seed Extract and Amoxicillin

RESULT AND DISCUSSION:

Fabrication of all patches successfully worked subjected to diffusion study which is carried out with the help of the Franz diffusion cell method and Everted Gut Sac model. Samples were collected at predetermined time and absorbance of every sample was measured with the help of spectrophotometer in order to find out the %of drug content. The result of the diffusion studies has been discussed in graph by plotting time in X axis and cumulative % release in Y axis as well as % absorbance against time in case of Everted Gut Sac Model. During this study it has been found that natural bioenhancers like Black Cumin seed extract can be used along with modern medicine like Amoxicillin in order to improve bioavailability of drug

In Cumin seed extract along with Amoxicillin the Methanolic extract showed significant increase in % CDR

1. Compatibility studies of drug and extract as well as drug and polymers were studied with the help of FTIR shows no drug extract and drug polymer interaction, result of which shown in **Figure 7-12**
2. Physicochemical parameters like % moisture content, thickness, weight variation etc. are within limit shown in **table 4**
3. Ex vivo permeability studies are mention in **table 5 and Figure 13**
4. Everted Gut Sac studies are mention in **table 6 and Figure 14** Amongst all the extract Methanolic extract of Black Cumin Seed (F25) showed significant increase in % CDR as well as in drug absorbance.

As an extension to this work In-vivo studies and clinical research on human being can be carried out in future.

CONCLUSION: It can be concluded that herbal drugs in the form of extract can also be used in formulating transdermal patches due to opportunity of release of drug formulation which is very novel approach.

The Amoxicillin patches made by solvent evaporation technique comprising of different extract of Black cumin seed, with Amoxicillin were formulated. The drug was found compatible different extracts and the polymers. All extracts shows to some extent bioenhancing effect compared to individual Amoxicillin patch. Amongst all the formulations F25 showed significant increase in drug release and drug absorption.

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CONFLICTS OF INTEREST: Authors have no conflict of interest regarding this research work.

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