RESEARCH ARTICLE

Phytochemical Screening, Characterization and Formulation and Evaluation of Herbal Gel of *Abelmoschus Manihot* for Atopic Dermatitis

Pankaj Singh Chauhan*, Dr. S. S. Sisodia[1]

**Department of pharmacy, Research scholar, B. N. University, Udaipur 313001 Rajasthan*
[1]Department of pharmacy, Professor and Head, B. N. University, Udaipur 313001 Rajasthan

Received: 5th Nov, 2024; Revised: 1st Dec, 2024; Accepted: 15th Dec, 2024; Available Online: 25th Dec, 2024

ABSTRACT
Atopic dermatitis is skin disease that is commonly known as eczema that causes dry, itchy and inflamed skin it is irritating disease but cannot spread from one person to another through direct or indirect contact. Literature review also reveals treatment of Atopic Dermatitis (AD) have been reported for the extracts and active compounds from *A. manihot* both *in vitro* and *in vivo.* From the MIC concentration has been proved the ethanolic extract was *in-vitro* potential for Atropic dermatitis activity, Isolated compound is positively to the Salkowski's test and Lieberman-Burchard test for steroids and triterpenes. Evaluation of formulated gel by different parameters have been done Physiochemical parameters like appearance, consistency and homogeneity were determined by visual inspection. *In vitro* diffusion study carried out in diffusion cell for 4 hr showed HG III with maximum drug release (87.25 %) as compared to the other gel formulations. *Ex-vivo* permeability and retention studies done In terms of drug penetration, both th systems were showing almost comparable results; however retention was higher with HG-II gel compared to the HG-III gel.
Keywords: Atopic dermatitis; Herbal Gel; eczema; AD and Xerosis.
How to cite this article: Pankaj Singh Chauhan, S. S. Sisodia. Phytochemical Screening, Characterization and Formulation and Evaluation of Herbal Gel of Abelmoschus Manihot for Atopic Dermatitis. International Journal of Pharmaceutical Quality Assurance. 2024;15(4):2396-404. doi: 10.25258/ijpqa.15.4.38
Source of support: Nil.
Conflict of interest: None

INTRODUCTION

Atopic Dermatitis (AD) Atopic dermatitis (AD) is commonly occurring and complex disease. It is chronically relapsing and exhibits immunological abnormalities in the skin. AD is a potentially enervating skin disease which demonstrates a substantial influence on mankind worldwide. The diagnosis of AD is done by characteristic features like erythematous plaques, eruption, flexural lichenification, intense pruritus and cutaneous hypersensitivity reactions (Gaspar and Aidé). Cytokines and chemokines are released in the skin as a result of scratching and mechanical injury due to intense pruritus. It promotes the skin permeability and entry of allergens in the skin.

The researchers are unsure about the pathogenesis of AD for decades. There is still a lacuna in revealing consolidated proof of concept of AD [1]. By elucidating the mechanism of occurrence of AD in inherently susceptible individuals, researchers have already achieved a salient milestone. Histology reveals the presence of eosinophils, macrophages and T cells in case of relapsing itch and inflammation (observed as overgrowth of dermis and epidermis) in AD.

Interestingly, in AD immunological imbalance and skin barrier abnormality present a cause and effect dilemma. The skin becomes more susceptible for the internalization of numerous allergens due to disturbed epidermal barrier function and dryness, along with other adverse environmental factors. This action triggers and provokes allergy with one more episode of inflammation and barrier integrity alteration (Leung et al.; Scharschmidt and Segre). Therefore, dry skin is one of the most prominent factor in damaging the skin and also contributes in pathogenesis of AD (Bouwstra, de Graaff, et al.). It could be said that barrier function restoration is essential while designing prevention plan and aggravation of AD.

Medicinal plants are not only used for primary health care and not just in rural areas in developing countries, but also in developed countries as well where modern medicines are predominantly used [2]. In western world also, the use of herbal medicines is steadily growing with approximately 40 percent of population reporting use of herb to treat medical illnesses in 2004 [3]. Public,

Figure 1: *Abelmoschus manihot* plant

**Author for Correspondence*

Table 2: Composition of Herbal Gel (HG)

S.N.	Ingredients	P-I		
		HG I	HG II	HG III
1	Extracts*	1%	2%	3%
2	Menthol	1%	1%	1%
3	Linseed oil	1%	1%	1%
4	HPMC	2%	2%	2%
5	DMSO	2%	2%	2%
6	Triethanolamine	1.5%	1.5%	1.5%
7	Methyl Paraben	0.5%	0.5%	0.5%
8	Methanol-Water mixture	Q.S.	Q.S.	Q.S.

*isolated compounds from ethanolic extract of *Abelmoschus manihot* [P-I]

academic and government interest in traditional medicines is growing exponentially due to the increased incidence of the adverse drug reactions and economic burden of the modern system of medicine [4].

MATERIALS AND METHODS

Entire plants of *Abelmoschus manihot* was collected from agriculture region of Rajsamand district. The plant was authenticated by Dr. K.B. Sukla (botanist) Principal, Shrinathji Agriculture College, Nathdwara. A voucher specimen is preserved in our laboratory for future reference (Voucher No.: DSPSC/2022-23/012).

Preparation of plant material

The Leaves of plant were shade dried, reduced to coarse powder with the help of grinder and stored in airtight container till further use [5]. The leaves of *Abelmoschus manihot* plants were shade dried, reduced to coarse powder with the help of grinder and stored in airtight container till further use.

Determination of Water Soluble Extractive Value

Weigh accurately the 10 gm of coarsely powdered drugs and macerate it with 100 ml of water in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing standing for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Then 25 ml of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish, dried at 105°C and weighed [6]. The percentage of water soluble extractive was calculated with reference to the air dried drugs.

Loss on Drying

About 1.5 gm, of powdered drug was weighed accurately in a porcelein dish which was previously dried at 105°C in hot air oven to constant weight and then weighed. From the difference in weight, the percentage loss of drying with reference to the air dried substance was calculated.

Determination of Foreign Organic Matter

WHO indicates that restorative plant material ought to be liberated from any perilous or harmful unfamiliar issue and beyond what many would consider possible liberated from harmless unfamiliar issue. Unfamiliar issue was resolved according to Pharmacopoeial necessity (Anonymous, 2001). Leaves powder of *Abelmoschus manihot* (Approximately 250g) were independently spreader out in a slim layer over white piece of paper and

Table- 3: Phytochemical screening of extracts of *Abelmoschus manihot*

Chemical Constituents	Chemical Test	Extracts/Fractions						
		Ethanol extract	Aqueous extracts	n-hexane fraction	Chloroform fraction	Ethyl acetate fraction	Ethanol fraction	Aqueous fraction
Alkaloids	Mayer's	+	-	+	+	+	+	-
	Dragendorff's	+	-	+	+	-	+	-
Saponin	Foam forming test	+	+	-	-	+	-	-
Tannins	Ferric Chloride	+	+	+	+	-	+	-
	Dilute nitric acid	+	+		-	+	+	+
Proteins	Million's	-	+	-	+	+	-	+
	Biuret	-	+	-	+	-	-	+
Flavonoids	Shinoda	+	+	+	-	-	-	+
	Lead Acetate	+	+	-	-	-	-	+
Glycoside	Killer killani	-	+	-	-	-	-	-
Carbohydrate	Molisch's	+	+	-	-	+	-	-
	Fehling's	+	+	+	+	-	-	+
Triterpenes	Vanillin-sulphuric acid test	-	-	-	-	-	-	-
Amino Acids	Ninhydrin	-	+	-	-	+	-	+
Sterols	Liebermann-Burchard's	+	-	-	+	-	+	-
	Salkowski's	+	-	-	+	-	+	-
Phenol		+	+	+	+	+	+	+

Key (+) = Presence, (-) = Absent

Table 4: The extractive values of various extract of leaves of *Abelmoschus manihot*

Sr. No.	Extracts/ Fractions	Estimated percentage	Colour of extract
1.	Ethanol extract	9.45 % w/w	Dark green
2.	Aqueous extracts	11.21% w/w	Dark Brown
3.	n-hexane fraction	3.73 % w/w	Greenish brown
4.	Chloroform fraction	4.52 % w/w	Dark Yellow
5.	Ethyl acetate fraction	4.02 % w/w	Light Brown
6.	Ethanol fraction	19.14 % w/w	Light green
7.	Aqueous fraction	21.08 % w/w	Light yellowish

Table 5: Percentage of phytoconstituents present in *Abelmoschus manihot*

Extracts/ Fractions	Constituents presents	Quantity of phytoconstituents in (%)
Ethanol extract	Alkaloids	16.12
	Phenols	6.12
	Flavonoids	08.14
	Tannin	07.52
Aqueous extracts	Alkaloids	5.17
	Phenols	0.00
	Flavonoids	09.16
	Tannin	06.20
n-hexane fraction	Alkaloids	08.74
	Phenols	4.11
	Flavonoids	05.42
	Tannin	06.56
Chloroform fraction	Alkaloids	10.25
	Phenols	5.14
	Flavonoids	0.00
	Tannin	04.16
Ethyl acetate fraction	Alkaloids	07.63
	Phenols	3.19
	Flavonoids	0.00
	Tannin	04.03
Ethanol fraction	Alkaloids	14.08
	Phenols	6.22
	Flavonoids	0.00
	Tannin	6.18
Aqueous fraction	Alkaloids	0.00
	Phenols	4.87
	Flavonoids	6.06
	Tannin	3.28

examined with the independent eye and isolated the unfamiliar issue by hand as complete as could be expected under the circumstances [7]. The dried powder was gauged and level of unfamiliar natural issue was resolved from the heaviness of the powder taken.

Extraction and fractionation

The extraction yield of the extracts from plant species is vastly depends on the solvent polarity, which find out both qualitatively and quantitatively the extracted compounds. Ethanol and water are the commonly used solvent for the extraction because of their low toxicity and high extraction yield with the advantage of modulating the polarity of the solvent by using mixtures at different ratios (Jackson et al, 1996). The plant materials (1 kg) were initially defatted with petroleum ether and then extracted with n-hexane (E1), chloroform (E2), ethyl acetate (E3), alcohol (E4) and water (E5) using a Soxhlet apparatus. The yield of the plant extracts ethanol (70%) and aqueous measured about 20 g each after evaporating the solvent using water bath. The standard extracts obtained from *Abelmoschus manihot was* then stored in a refrigerator at 4°C for further use for phytochemical investigation and pharmacological screening [8].

Qualitative examination of phytoconstituents were performed by identification test for Alkaloids, Carbohydrates, Glycosides, Phenolic Compounds, Steroids, Triterpenoids, Flavonoid and Saponins.

Quantitative determination of the chemical constituents for Total phenols Determination, Total alkaloid determination, Total tannin determination and Total flavonoid determination were performed as per standard procedure.

Thin layer chromatography (TLC)

The effectiveness of the separation depends on the mixture to be separated, the choice of the mobile phase and the adsorption layer and the term retention factor R_f, is commonly used to describe the chromatographic behavior of sample solutes. The R_f value for each substance is the distance it has moved divided by the distance the solvent front has moved. The solvents, during this research for each crude extract, were chosen by trial and error. The selection was made on the basis of best resolution. The different solvents system has been used of TLC.

Optimization of solvent system

Ethanol soluble fractions were analyzed by TLC. These fractions constituted of mainly non-volatile mixtures of compounds. The visualizations were aided by either observing the TLC under an UV lamp or by exposing the developed TLC plates to iodine vapour [9]. The TLC was repeatedly improved by changing the solvent systems until a system that gave the best separation was obtained.

Elution procedure

There are three principle elution procedure commonly employed. They are isocratic elution, stepwise elution and gradient elution. Isocratic elution involves the operation of chromatographic column by allowing a solvent mixture of unvarying composition to run through the column until separation is complete. Stepwise elution involves generally if only one solvent is used, elution of only some of the components of the mixture results. Hence to remove the components, which are firmly held, a stronger eluting solvent will be required. Gradient elution technique was first described by Williams and Tiselius which involves the use of a continuously changing eluting medium [10].

Collection of eluting sample

Elute was collected at the rate of 20 drops per minute and each fraction was of about 100 ml. Each fraction was

Table 6: TLC Studies of ethanolic extract, aqueous extract, n-hexane fraction, chloroform fraction, ethyl acetate fraction, ethanolic fraction, aqueous fraction of *Abelmoschus manihot*

Fraction/ Extract	Solvent system	No of spots	TLC profile	
			R_f value	Color
Ethanol extract	Toluene:Ethyl acetate:Metenol: Water(7:6:5:2)	9	0.94;0.90;0.81; 0.74;0.69;0.61; 0.59;0.45;0.38	Dark green, green, green, faint green ,pale green, yellow , light yellow, light green, brown
Aqueous extracts	Ethyl acetate: methanol: toluene: water (5:4:6:5)	5	0.90;0.86;0.73; 0.54;0.32	Dark green, green, light green, light yellow, brown
n-hexane fraction	Hexane: methanol (7:3)	3	0.57; 0.40;0.38	Light brown , light yellow , light brown
Chloroform fraction	Hexane: Ethyl acetate: methanol (10:3:7)	5	0.70;0.64;0.58; 0.47;0.39	Light green, Very light green, Light brown , light yellow , light brown
Ethyl acetate fraction	Hexane:DCM: Ethyl acetate: Methanol (10:5:2:3)	6	0.76;047;0.41 0.29;0.27;0.22	Dark yellow, Dark brown, Dark brown, Very light green. Light yellow, light brown
Ethanol fraction	Toluene:Ethyl acetate:Metenol: Water(7:6:5:2)	1	0.94	Dark brown
Aqueous fraction	Ethyl acetate: methanol: toluene: water (5:4:6:5)	1	0.90	Brown

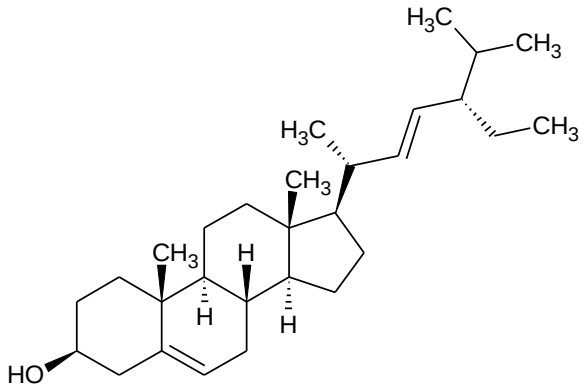


Figure 2: Structure of Stigmasterol isolated from ethanol fraction of *Abelmoschus manihot*

subjected to TLC on silica gel G. The fractions with same R_f were pooled together and concentrated to obtain pure compounds.

Isolation of compound from chloroform fraction of *Abelmoschus manihot*

The dried chloroform fraction of *Abelmoschus manihot* (20 gm) was mixed with 80 gm silica gel (60-120 mesh) to make the material to get adsorbed in the silica gel. The column was eluted with solvent increasingly initial from 100 % n-hexane, than increasing order of ethyl acetate in n-hexane (0, to 100% ethyl acetate in n-hexane) and total 108 fractions were collected. After evaporating the solvents on water bath all the collected fractions were subjected to TLC analysis. On the basis of Rf values, same fractions were pooled together [11]. The pools which gave single spot in iodine exposure were 8-19, 28-35, and 74-86. On the basis of high quantity, 74-86 pool of fractions was taken for purification.

Purification of the isolated compound from *Abelmoschus manihot*

Fractions from column chromatography were subjected to preparative TLC as required to obtain pure compounds. Mixed fractions of 74-86, after evaporating solvent, was subjected for thin layer chromatographic study using various solvent systems. Among them the Toluene: Acetone (8:2) gave good resolution this solvent system was further selected for preparative TLC. The concentrated pool was dissolved in chloroform and the sample was spotted in the preparative TLC plates. The sample applied plates were kept in completely saturated chamber of selected mobile system. After development of chromatogram, the plates were put in iodine vapour and the band (Rf =0.64) was identified and scrapped out from the plates. The scrapped material was then dissolved in pet ether and filtered through whatman filter paper [12]. The filtrate was concentrated and the isolated product was obtained as white solid powder (64 mg).

Characterization of isolated compounds by spectral analysis and structural elucidation

Tools most widely used for structure elucidation of natural products are Fourier transform infrared spectroscopy (FTIR), mass spectroscopy (MS) and nuclear magnetic resonance (NMR). With these tools the structures of most natural products can be determined. FTIR identifies chemical bonds in a molecule fingerprint that can be used

Table 7: Physicochemical parameters of formulated herbal gel

Formulation Code	Appearance	Consistency	Homogeneity
HG I	Transparent with light brown color	Smooth	Amorphous
HG II	Transparent with brown color	Mild Smooth	Amorphous
HG III	Dark brown color	Highly Smooth	Amorphous

to easily screen and scan samples for many different components. It detects functional groups and characterizes covalent bonding information. NMR has become increasingly more important in structure elucidation. It has almost completely replaced degradation studies in the determination of novel structures, and in many cases synthesis is no longer necessary as a structural proof. The ^{1}H NMR produces spectrum produced by signals that indicate the number of different types of protons and the integration specifies the ratios of number of protons. This information actually provides information concerning the number of different types of hydrogen present in the molecule, the relative numbers of the different types of hydrogen, the electronic environment of the different types of hydrogen and the number of hydrogen "neighbour" hydrogen has. NMR experiments are very commonly used in natural product chemistry. In most cases they are capable of providing molecular structures without the need of further experimentation. The integration of these molecular spectroscopic techniques provides complementary data regarding a molecule's structure, and when used together they prove very effective in identification of unknown compounds (Silverstein, 2006; Pavia et al, 2008). With these tools the structures of most natural products can be determined [13].

UV spectra of the isolated compounds were recorded in methanol over a scanning range of 200-400 nm and λmax of compounds were determined. Spectra were recorded with a Shimadzu 1700 double beam- UV-VIS spectrophotometer. EIMS (electron impact mass spectrum) in positive mode were recorded on Waters Micromass Q-Tof Micro mass spectrometer instrument at SAIF, Chandigarh. The isolate was mixed with 200 mg KBr (FT-IR grade) and pressed into a pellet. The sample pellet was placed into the sample holder and FT-IR spectra were recorded in the range 375- 7500 cm-1 in FT-IR spectroscopy (Model RZX (Perkin Elmer) at SAIF, Chandigarh. ^{1}H and ^{13}C-NMR spectra were recorded on a FT-NMR Cryomagnet Spectrometer 400 MHz (Bruker) using TMS as an internal standard at SAIF, Chandigarh, India. The solvents used were methanol and DMSO. Chemical shifts ware shown in δ values (ppm) with TMS as an internal reference. For column chromatography silica gel 60 (70-230 mesh, Merck, Darmstadt, Germany) was used. Solvents for chromatography were distilled before use. Thin layer chromatography (TLC) was performed using TLC plates (Silica Gel G-60).

Formulation of Herbal Gel (HG)

Gel was prepared by cold mechanical method described by Lalit K et al, 2010. Step 1- Required quantity of polymer (HPMC) was weighed and it was sprinkled slowly on surface of purified water for 2 hrs. After which it was continuously stirred by mechanical stirrer, till the polymer soaked in the water. Step 2- Triethanolamine was added with continuous stirring to neutralize the gel and it maintains the pH of the gel. Then the appropriate quantity of DMSO (Dimethyl sulfoxide) was added to the gel, which behaves as the penetration enhancer, followed by the required quantity of methyl paraben as a preservative. Step 3- Finally the *Abelmoschus manihot* extracts, Menthol, Linseed oil were added to the gel with continuous stirring till all the ingredients get dispersed in gel completely [14, 15]. The quantity of each formulation material is mentioned below;

The above formula is selected on the basis of literature available for the formulation of herbal gel with these constituents

Evaluation of Formulated Gel by Different Parameters

The herbal gel is formulated with isolated fraction [P-I], menthol, linseed oil and isolated compounds from ethanolic extract of *Abelmoschus manihot* [P-I] with various excipients. The formulated herbal gel (HG) is evaluated in terms of various physicochemical parameters, pH, Viscosity, extrudabilty and spreadibility.

Measurement of pH

The pH of developed gel formulations was determined using digital pH meter. 1 gm of gel was dissolved in 100 ml distilled water and kept aside for two hours. The measurement of pH of each formulation was done in triplicate and average values are calculated [16].

Determination of Viscosity

The measurement of viscosity of the prepared gel was done with a Brookfield Viscometer. The viscosity of the gel was obtained by multiplication of the dial reading with factor given in the Brookfield Viscometer catalogues. All the findings were calculated and recorded [17].

Extrudability

The gel formulations were filled in standard capped collapsible aluminum tubes and sealed by crimping to the end. The weights of the tubes were recorded. The tubes were placed between two glass slides and were clamped. 500 gm was placed over the slides and then the cap was removed. The amount of the extruded gel was collected and weighed. The percent of the extruded gel was calculated (>90% extrudability: excellent, >80% extrudability: good, >70% extrudability: fair) [18].

Spreadability

Spreadability was determined by the apparatus which consists of a wooden block, which was provided by a pulley at one end. By this method spreadability was measured on the basis on slip and drag characteristics of gels. An excess of gel (about 2 gm) under study was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the

hook. A one kg weighted was placed on the top of the two slides for 5 min. to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected topull of 80 gm. With the help of string attached to the hook and the time (in sec.) required by the top slide to cover a distance of 7.5 cm be noted [19]. A shorter interval indicates better spreadability. (Jadhav KR, 2010). Spreadability was calculated using the following formula**:**
S= M × L/ T
Where, S= Spreadability, M= weight in the pan (tied to upper slide),L= Length moved by the slide, T= Time (in sec.)

***In vitro* diffusion study**

The diffusion studies of the formulated herbal gels were carried out in franz diffusion cell for studying the dissolution release of gels through a cellophane membrane. Gel sample (1gm) was taken in cellophane membrane and the diffusion studies carried out at 37±1ºc using phosphate buffer as dissolution medium. Five ml of each sample was withdrawn periodically at 30, 60, 90, 120, 150, 180, 210, 240 minutes and each sample was replaced with equal volume of dissolution medium. The samples were analyzed for the drug content by using distilled water as blank (Negi A *et al*, 2012).

Pharamcological Evaluation for Atopic Dermatitis Activity

***In-vivo* efficacy study:**

The following groups of animals were used for the present study. The rats were divided into Six groups (n=6 in each group) of similar weight. A total of 36 animals for plant extract formulation. The rats were divided into the following 6 groups of mice each: Group I: Served as normal control, Group II: mice were sensitized initially by topical application of 0.5 % w/v dinitrofluorobenzene (DNFB), Group III: mice were treated with at Standard gel formulation (German's Graphites Eczema Cream 25gm), Group IV: mice were treated with at lower dose 10mg/kg Herbal gel formulation (HG-I), Group V: mice were treated with at medium dose 20mg/kg Herbal gel formulation formulation (HG-II), Group VI: mice were treated with at higher dose 30mg/kg Herbal gel formulation formulation (HG-III).

All animal experiments were conducted as per institutional ethics approval. Disease was induced in adult BALB/c mice of mix gender (n=6 in each group). Following a week of acclimatization, hapten induction method was used to induce AD on the ears of mice (Jin et al.). Skin was sensitized initially by topical application of 0.5 % w/v dinitrofluorobenzene (DNFB) solution prepared in vehicle of acetone:olive oil in the ratio of 80:20. Animals were challenged with application of 20 µl, 0.2 % w/v DNFB solution on both the sides of the ears, after 5 days of sensitization to induce inflammation. This was repeated every alternate day for 2 weeks to induce The response and develop severe disease phenotype mimicking human AD. (Petersen). The treatment was started on day 21 of the disease induction and animals were treated with obtained fractions from extracts for 7 days (till the animals cured completely). Response to the treatments for AD was observed by grading skin reactions and measuring ear thickness using a digital micrometer [20]. The efficacy was assessed by calculating improvement index of both the formulations with respect to the placebo. These results were also confirmed histologically via hematoxylin and eosin (H&E) staining.

Statistical analysis

The data were expressed as mean ± SEM. All the data were analyzed by one way analysis of variance (ANOVA) followed by "Dunnet's t-test." *p*-value less than 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Physiochemical analysis of crude drug

The total ash value was found to be 8.27% w/w indicating the considerable presence of inorganic radicals. The acid insoluble ash was found to be 2.43 %w/w. the difference between the total ash and acid insoluble ash indicates that the ash of leaf powder contains considerable amount of inorganic radicals like calcium oxalate which are acid insoluble. The water soluble ash value was found to be 11.21w/w. The water soluble ash value was found to be

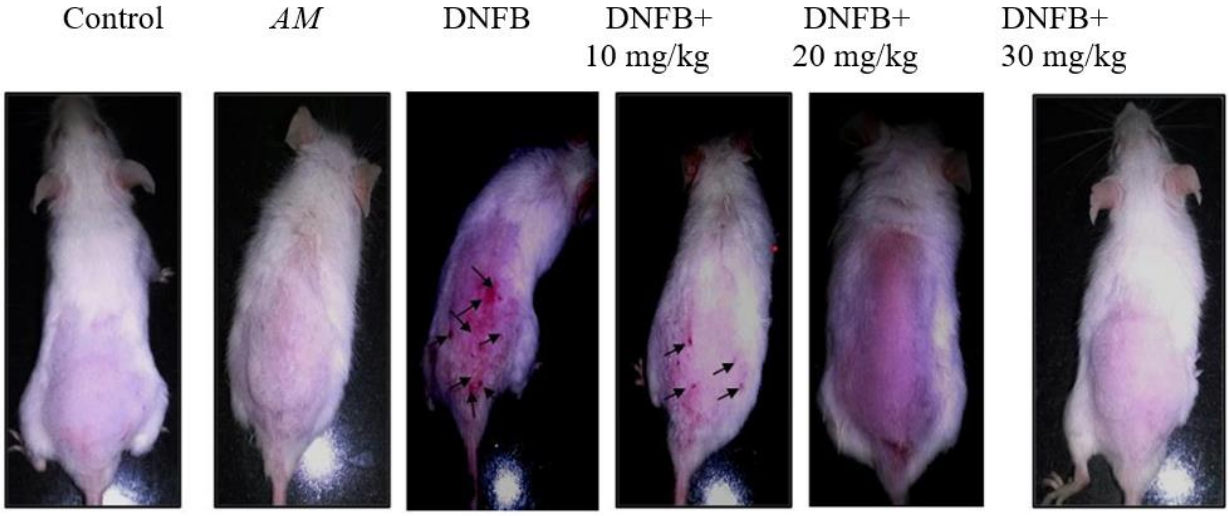
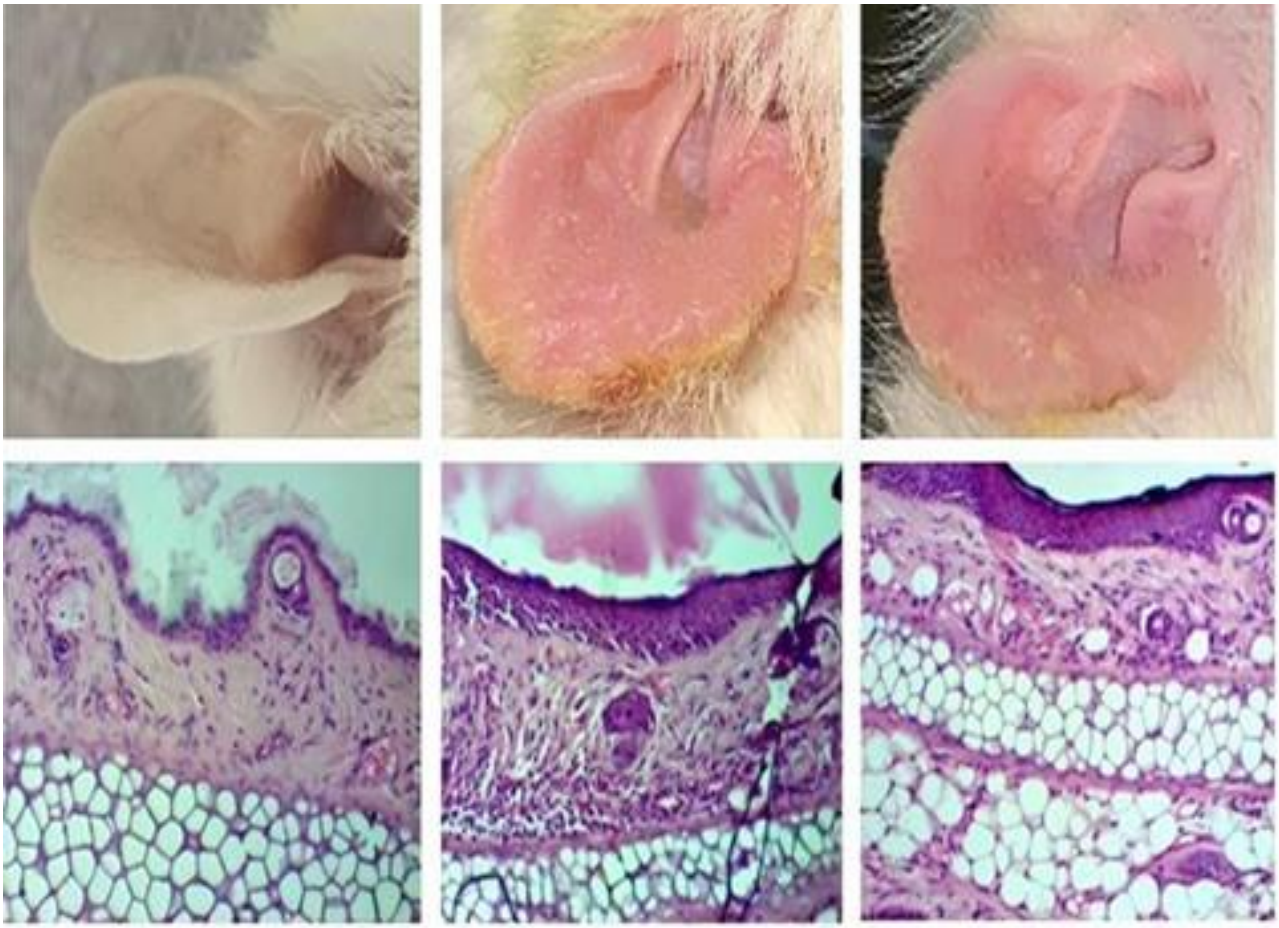


Figure 3: ***In vitro* Drug Diffusion study**



Normal control Disease control Treated with HG-III Gel

Figure 4: *In-vivo* efficacy results and their histological evaluation for HG-III gel

2.17%w/w. and the moisture content was found 4.72%.Percentage yield of all extract of leaves of *Abelmoschus manihot* were determined. The alcohol soluble and water soluble extractive values were found to be 6.35% w/w and 11.21% w/w respectively. It shows that water soluble extract having higher constituents as comparison to alcohol soluble extract. The moisture content of the drug was determined by the Loss on drying (LOD) method. It was found to be 4.72% w/w.

Isolation of active constituent from the ethanolic extract

The isolation was started with hexane and polarity increased with ethyl acetate. The elution up to hexane: ethyl acetate (5:5), were mixed together on the basis of their TLC profile to get fraction (P-I). This fraction was concentrated and rechromatographed with hexane DCM mixture with increasing proportion of DCM. The elution up to hexane: DCM (7:3) were mixed together and refrigerated overnight with addition of di-ethyl ether, which yielded compounds (P-I) as a white powder, which was recrystallized as yellow mass with acetone

Characterization of isolated phytoconstituent from ethanol fraction of *Abelmoschus manihot* using spectroscopic techniques

The various absorption spectrums of the isolated compound from chloroform fraction of *Abelmoschus manihot* showed peaks as follows:

Compound A **:** Isolated from ethanolic fraction
Synonym : Stigmasta-5, 22-dien-3β-ol
Molecular formula : $C_{29}H_{48}O$
Molecular weight : 411.69 g/mol
Description : white amorphous solid
Solubility : Soluble in Petroleum ether and Chloroform
R_f value : 0.63
M. P. : 169-170^{0}C
Phytochemical Test : The compound gave a red colour for Salkowski‟s test while a green colour in case of Liebermann- Burchard‟s tests.

5.9.2. Spectroscopic data

UV (λ_{max}) : 257 nm [Petroleum ether (60-80)]
IR (ranges in cm^{-1}) : 3428 (O-H stretching), 2937(C-H stretching), 2852(C-H stretching), 1642 (C=C stretching), 1465 (C-H bend.), 1460 (C-H bend.), 1192 (O-H bend.), 1053 (C-C str.), 739 (CH_2 rocking), 699 (O-H bend.).

^{1}HNMR (DMSO) : δ 5.24 (m, 1H, H-6), δ 4.57 (s, 1H), δ 4.14 (s, 1H), 3.20 (tdd, OH, H-3), δ 1.23 (s, 3H), δ 1.19 (s, 3H), δ 1.06 (s, 3H), δ 0.98 (s, 3H), δ 0.91 (s, 3H).
^{13}CNMR (DMSO) : δ 140.8 (C-22), δ 130.1 (C-5), δ 129.1 (C-23), δ 121(C-6), δ 71.6 (C-3), δ 56.1 (C-4), δ 55.1 (C-5), δ 52.2(C-24), δ 50.10, (C-17), δ 43.8 (C-9), δ 41.2 (C-13), δ 39.4 (C-10), δ 37.7, (C-10), δ 33.4 (C-20), δ 31.7 (C-25), δ 29.1 (C-21), δ 28.1 (C-23), δ 25.1 (C-12), δ 21.8 (C-11, C-25, C-26), δ 15.1 (C-29), δ 12.8(C-27).
EI MS : 412 [M+, $C_{29}H_{48}O$] 355(101), 311 (49), 301 (49), 279 (71), 219 (60), 200 (65), 175 (95)
Isolated compound is positively to the Salkowski's test and Lieberman-Burchard test for steroids and triterpenes. The melting point of compound AM was 169°C; the UV λ_{max} value of compound AM was 257 nm. Mass spectrum of isolated compound AM showed parent molecular ion [M^+] peak at mlz 412 which corresponds to the molecular formula $C_{29}H_{48}O$.
In the IR spectrum of isolated compound a very intensely broad peak at 3428 cm^{-1} and moderately intense peak at 1192 and 699 cm^{-1} were observed for the O-H bond vibrations of hydroxyl group. In the ^{1}H-NMR spectrum of isolated compound, H-3 proton appeared as a triplet of a double doublet (tdd) at δ3.20 and, H-6 olefinic proton showed a multiplet at δ5.24. Two olefenic protons appeared downfield at δ 4.57 m and δ 4.14 m. Six methyl protons also appeared at **δ** 1.23, **δ** 1.19, **δ** 1.06, **δ** 1.00**, δ** 0.98 and **δ** 0.91 (3H each, s, CH_3).
The ^{13}C-NMR has shown recognizable signals at 140.8 and 121 ppm, which corresponds to double bond at C-22 and C-6 double bonds respectively as well as it also represent signals at 130.1 and 129.1 ppm, which shows one more double bond in between C-5 and C-23. The δ value at 71.6 ppm is due to C-2 β- hydroxyl group. The signal at δ 31.7 and δ 12.8 ppm corresponds to angular carbon atom at C-25 and C-27 respectively.

Evaluation of Formulated Gel by Different Parameters

Physiochemical parameters like appearance, consistency and homogeneity were determined by visual inspection and the findings were calculated and recorded in table. The formulation HG I was smooth in consistency, amorphous and Transparent with light brown color.

pH Measurment

The measurement of pH of each prepared formulation was done in triplicate and average values are calculated and recorded The pH of HG I was found 5.8.

Viscosity

The viscosity of the formulated herbal gel was obtained by multiplication of the dial reading with factor given in the Brookfield Viscometer catalogues. The viscosity of HG I was found 23800 (cps).

Extrudability

The amount of the extruded etoricoxib herbal gel of was collected and weighed. The percent of the extruded gel was calculated. The extrudability of all three formulations were 74% for HG I. Findings were shown the possibility of preparing the formulation more satisfactory.

Spreadability

Spreadability was measured on the basis on slip and drag characteristics of formulated etoricoxib herbal gel. Spreadability of formulated herbal gel was found 18.8 for HG I.
On the basis of various evaluation parameters such as pH, viscosity, spreadability and extrudability the formulation HG-III for was selected for further the pharmacological evaluation parameters.

***In vitro* Drug Diffusion study**

In vitro diffusion study carried out in diffusion cell for 4 hr showed HG III with maximum drug release (84.00 %) as compared to the other gel formulations. Based on the studies on various inflammatory conditions, we determined the effect of *Abelmoschus manihot* at doses, 10, 20 and 30 mg/kg b.wt. First, to analyze the effect of *Abelmoschus manihot* on the phenotypic changes associated with AD in mice [21], *Abelmoschus manihot* was administered daily during the last sensitization phase to the dinitrofluorobenzene (DNFB) exposed mice.

Evaluation of formulated herbal gel for atropic dermatitis activity

***In-vivo* efficacy studies:**

In-vivo efficacy studies indicate that though HG-V is more promising in terms of solubility and *in-vitro* drug release studies, both the systems are showing equal response for reduction of the inflammation in mice ears. Histological examination also confirmed that neutrophils infiltration and thickness of epidermal layer reduced equally with HG-III gel. These results were different than the anticipated results from other comparative studies. These studies are preliminary confirmation, however, detailed studies using *in-vivo* models and other responsible inflammatory markers in AD can be studied in detail**.**

CONCLUSION

In vitro diffusion study carried out in diffusion cell for 4 hr showed HG III with maximum drug release (87.25 %) as compared to the other gel formulations. *Ex-vivo* permeability and retention studies done In terms of drug penetration, both th systems were showing almost comparable results; however retention was higher with HG-II gel compared to the HG-III gel. Hence it was concluded that the amorphous system is showing better results. Histological examination also confirmed that neutrophils infiltration and thickness of epidermal layer reduced equally with HG-III gel. These results were different than the anticipated results from other comparative studies.

REFERENCES

1. Desai AR, Vijapur LS, Teli CA, Terdal S. formulation and evaluation of herbal anti-microbial cream containing hibiscus abelmoschus linn extract.
2. Dos Santos DS, Barreto RD, Serafini MR, Gouveia DN, Marques RS, de Carvalho Nascimento L, de Carvalho Nascimento J, Guimaraes AG. Phytomedicines containing Matricaria species for the treatment of skin diseases: A biotechnological approach. *Fitoterapia* 2019; **1**:104267.
3. Duan X, Li J, Cui J, Li H, Hasan B, Xin X. Chemical component and in vitro protective effects of *Matricaria*

chamomilla (L.) against lipopolysaccharide insult. *Journal of Ethnopharmacology* 2022;**5**:115471.
4. El Joumaa MM, Borjac JM. Matricaria chamomilla: A valuable insight into recent advances in medicinal uses and pharmacological activities. *Phytochemistry Reviews* 2022;**1**:1-28.
5. Simanjuntak SB, Kalalo MJ, Hebber T, Tallei TE, Fatimawali. Angiotensin converting enzyme inhibitors from Abelmoschus manihot (L.) Medik leaves: A molecular docking study. *In AIP Conference Proceedings* 2022;**18:** 70002.
6. Sun X, Li P, Lin H, Ni Z, Zhan Y, Cai G, Liu C, Chen Q, Wang W, Wang X, Li P. Efficacy and safety of Abelmoschus manihot in treating chronic kidney diseases: A multicentre, open-label and single-arm clinical trial. *Phytomedicine* 2022; **1**;99:154011.
7. Kwon HJ, Beom SH, Hyun JA, Kang EB, Park HE, Han DG, Choi EY, An BJ. Analysis of antioxidant activity, total phenol content, and flavonoid content of *Abelmoschus manihot* flower extracts. *Korean Journal of Food Preservation* 2022;**29**(1):157-65.
8. El Joumaa MM, Borjac JM. Matricaria chamomilla: A valuable insight into recent advances in medicinal uses and pharmacological activities. *Phytochemistry Reviews* 2022;**1**:1-28.
9. Yu JH, Geum NG, Ye JH, Jeong JB. Immuno-enhancing and anti-obesity effect of Abelmoschus manihot root extracts. *Korean Journal of Plant Resources* 2021; **1**;34(5):411-9.
10. Gul MZ, Bhakshu LM, Ahmad F, Kondapi AK, Qureshi IA, Ghazi IA. Evaluation of *Abelmoschus moschatus* extracts for antioxidant, free radical scavenging, antimicrobial and antiproliferative activities using *in vitro* assays. *BMC complementary and alternative medicine* 2011;**11**:(1):1-2.
11. Jain, N.K., 1997. Controlled and Novel drug delivery CBS Publisher and distributor, 1 st ed., 100-126.
12. Arora P, Shiveena B, Garg M, Kumari S, Goyal A. Curative Potency of Medicinal Plants in Management of Eczema: A Conservative Approach. *Phytomedicine Plus*. 2022; **11**:100256.
13. Li N, Tang H, Wu L, Ge H, Wang Y, Yu H, Zhang X, Ma J, Gu HF. Chemical constituents, clinical efficacy and molecular mechanisms of the ethanol extract of *Abelmoschus manihot* flowers in treatment of kidney diseases. *Phytotherapy Research*. 2021;**35**(1):198-206.
14. Park MH, Yeom YJ, Ganbat D, Kim MK, Kim SB, Lee YJ, Lee SJ. Fermentation of Abelmoschus manihot Extract with Halophilic Bacillus licheniformis CP6 Results in Enhanced Anti-Inflammatory Activities. *Nutrients* 2023;**7**:15(2):309.
15. Vyas, S. P., Khar, R. K., 2002. Controlled Drug Delivery: Concepts and Advances, Vallabh Prakashan, 1st ed., 411-447.
16. Sanjoy, M., Thimmasetty, j., Ratan, G. N.,Kilarimath, B. H., 2011. Formulation and evaluation of Crvedilol transdermal patches. Int. Res. J. Pharm. 2 (1), 237-248.
17. Jalwal, P., Jangra, A., Dahiya, L., Sangwan, Y., Saroha R., 2010. A review on transdermal patches. T. Ph. Res., 3, 139-149.
18. Kwon HJ, Beom SH, Hyun JA, Kang EB, Park HE, Han DG, Choi EY, An BJ. Analysis of antioxidant activity, total phenol content, and flavonoid content of *Abelmoschus manihot* flower extracts. *Korean Journal of Food Preservation* 2022;**29**(1):157-65.
19. Wu B, Zhou Q, He Z, Wang X, Sun X, Chen Y. Protective effect of the abelmoschus manihot flower extract on DSS-induced ulcerative colitis in mice. Evidence-Based Complementary and Alternative Medicine. 2021;**9**:2021.
20. Sun X, Li P, Lin H, Ni Z, Zhan Y, Cai G, Liu C, Chen Q, Wang W, Wang X, Li P. Efficacy and safety of Abelmoschus manihot in treating chronic kidney diseases: A multicentre, open-label and single-arm clinical trial. *Phytomedicine* 2022; **1**;99:154011.
21. Wang SW, Chang CC, Hsuan CF, Chang TH, Chen YL, Wang YY, Yu TH, Wu CC, Houng JY. Neuroprotective Effect of Abelmoschus manihot Flower Extracts against the H2O2-Induced Cytotoxicity, Oxidative Stress and Inflammation in PC12 Cells. *Bioengineering* 2022;**21**:9(10):596.