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FORMULATION, EVALUATION AND *IN VITRO* ANTIBACTERIAL SCREENING OF HERBAL GEL CONTAINING *MANILKARA HEXANDRA* (ROXB.) DUBARD LEAF EXTRACT

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ABSTRACT: *Manilkara hexandra* (Roxb.) [Synonym: *Mimusops hexandra*] belonging to family Sapotaceae is revealed to be endowed with antibacterial activity. Leaves, fruits, roots and barks of the plant are renowned for their medicinal values. Herbal gels have prevalence over conventional gels in piling up the therapeutic efficiency and narrowing down the side effects. The design of present study is to formulate and evaluate the herbal gel containing methanolic and hydro alcoholic leaf extract of *Manilkara hexandra* using varying concentration of carbopol 934 (1%, 1.25% and 1.5%). The physicochemical criterion of formulations like color, homogeneity, pH, viscosity, spreadability and drug content were determined. Diffusion studies were carried through Franz diffusion cell. Stability studies were accomplished as per ICH Guidelines for duration of 3 months. The result proffer that formulation with 1% gelling agent and 2.5% extract elicited better stability and henceforth optimized. Further optimized formulation was tested *in vivo*, for their skin irritability over a span of 7 days. The denouement claimed no skin irritation to the animals. The optimized gel was tested for antibacterial activity against different strains of bacteria such as *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Proteus vulgaris*. The results unveil good antibacterial potential of the gel.

INTRODUCTION: Traditional medicines gleaned from medicinal plants are used by about 80% of the world's population. ¹ Herbal formulations are preferred due to lesser side effects and low cost. ² Herbal drugs integrate a preponderance of all the officially recognized systems of health in India viz. Ayurveda, Yoga, Siddha, Homeopathy and Naturopathy, except Allopathy. ^{3,4}

Manilkara hexandra(Roxb.) Dubard (family-Sapotaceae) is a small to medium sized evergreen tree. It is native to South Asia and Tropical Countries and widely distributed in South, North and Central India-mainly in Rajasthan, Gujarat, Madhya Pradesh and Maharashtra. ⁵

Leaves, fruits, roots and barks of *Manilkara hexandra* are renowned for their medicinal values. ⁶ The leaf alcoholic and aqueous extracts of *Manilkara hexandra* when screened for antimicrobial property, were found to be efficacious against various strains of bacteria. ⁷

Infectious diseases are the leading cause of death worldwide. The clinical efficacy of many existing

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antibiotics is being jeopardized by the emanation of multidrug-resistant pathogens.⁸ The quiescent usage of herbal medicine to augment the antibiotics in order to increase the efficacy and or to prevent the bacterial resistance to the respective antibiotics.⁹ The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity.^{10,11}

There are several reports in the literature regarding the antimicrobial activity of crude extracts prepared from plants.¹² Topical preparations like gel offer better release of drug substances compared to creams and ointments. Gels often provide good compatibility with least adverse reactions. They are bestowed with many agreeable parameters like being thixotropic, emolient, non greasy, non-staining and are adaptable with several excipients.¹³

Aim of the present study is to formulate and evaluate novel herbal gel containing *Manilkara hexandra* leaves extract. The use of gel as a delivery system can increase the residence time of drugs on the skin and consequently enhance bioavailability. The topical gel was prepared by using different concentration of carbopol 934.

MATERIALS AND METHODS:

Collection of plant material: Fresh plant leaves of *Manilkara hexandra* (Sapotaceae) were collected from Tirumala Hills, Tirupati, Chittoor District, Andhra Pradesh, India in January 2016. Dr. K. Madhava Chetty (Department of Botany, Sri Venkateshwara University, Tirupati, India) identified and authenticated (Voucher Specimen No. 1257).

Extraction Procedure: The leaves of *Manilkara hexandra* were dried under shade, powdered and sieved through sieve No.14 and stored in air tight containers. The required quantity of dried powder

was taken in a round-bottomed flask and refluxed with methanol to obtain methanolic leaf extract of *Manilkara hexandra* (ME). Fresh solvent was added every one hour and all the extracts are combined to get the concentrate of ME by using rotavap. Analogous procedure was adopted using 60% methanol and 40% water to obtain hydroalcoholic extract of *Manilkara hexandra* (HAE).¹⁴

Chemicals: All chemicals used in this study are Analytical Reagent grade of Merck India Co. Ltd., and purified according to the standard procedures. Carbopol 934 was purchased from (Merck Ltd.), Methyl paraben, Propyl paraben and triethanolamine were purchased from SD Fine chemicals Ltd., Mumbai, India.

Micro-organisms: For the present study, the microbial strains were procured from Apollo Hospitals, Hyderabad, India. The microbial strains comprised of *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Proteus vulgaris*.

Formulation of Gel: Different proportions of carbopol 934 (1%, 1.25% and 1.5%) are dissolved in distilled water with continuous stirring. Dissolve 0.2ml of methyl paraben and 5ml of propyl paraben in distilled water by heating in separate beaker. Then add 2.5% leaf extract of *Manilkara hexandra* (ME and HAE) to the above solution and mix well.

This mixture is now added to carbopol solutions to obtain various formulations of the herbal gel containing methanolic and hydro alcoholic extracts of *Manilkara hexandra* (ME₁, ME₂, ME₃, HA₁, HA₂, HA₃) (**Table 1**). Triethanolamine was added drop wise to maintain the pH (6.8 - 7) and to obtain the gel of required consistency. Ethanol was added to increase gel permeation.¹⁵ Similar procedure was adopted to prepare control sample without *Manilkara hexandra* leaf extract.

TABLE 1: FORMULATION OF HERBAL GELS

Ingredient	ME ₁	ME ₂	ME ₃	HA ₁	HA ₂	HA ₃
Carbopol 934 (%)	1	1.25	1.5	1	1.25	1.5
Extract (% w/w)	2.5	2.5	2.5	2.5	2.5	2.5
Methyl paraben (0.5%) (mL)	0.2	0.2	0.2	0.2	0.2	0.2
Propyl Paraben (0.2%) (mL)	5	5	5	5	5	5
Ethanol (mL)	3	3	3	3	3	3
Triethanolamine (mL)	0.5	0.5	0.5	0.5	0.5	0.5
Distilled water	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S

Evaluation of Topical Gel Formulation:

Physical Evaluation: All gels were evaluated for colour, consistency and odour. They appear light greenish yellow and opaque, free from grittiness and homogeneously dispersed.

Measurement of pH: pH of the gel was measured by using digital pH meter. The measurement was done on the initial and final day of every month, for the span of three months.

Homogeneity: Inhabitancy of any aggregates in the gel formulations was visually inspected and the homogeneity was approved.

Spreadability: Spreadability was determined by the apparatus which consists of a wooden block, which was provided by a pulley at one end.¹⁶ By this method spreadability was measured on the basis of slip and drag characteristics of gels. An excess of gel (about 2 g) under study was placed on the ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with a hook. A 1 kg weight was placed at the top of the two slides for 5 minutes to dislodge air and to provide a uniform film of the gel between the slides. The excess gel was uncluttered from the edges. The top plate was then subjected to pull of 80 g with the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5 cm was noted. A shorter interval indicated better spreadability. Spreadability was calculated using the following formula:

$$S = M \times L / T$$

Where,

S = Spreadability

M = Weight in the pan (tied to the upper slide)

L = Length moved by the glass slide

T = Time (in sec.) taken to separate the upper slide from the ground slide.

Viscosity: Brookfield viscometer (Model DV-II+ Pro), 100 rpm with spindle no 6 was employed to measure the viscosity of all gel formulations. The calculations were made in triplicate by placing the required portion of gel in a beaker and impregnating the viscometer into the gel.¹⁷

Extrudability: Extrudability of the gel formulations was assessed by packing the gel in the aluminium tubes, folded at one end, and placing them in between two glass slides. The weight of 500 gms is placed over the slide and cap was unlocked. The volume of gel expelled is evaluated (Excellent extrudability: +++, Good extrudability: ++, Fair extrudability: +).¹⁸

Drug content: Drug content of the formulation was estimated by dissolving 500mg of gel in 60ml of distilled water and the volume is made up to 100ml. the contents are filtered through filter paper and analysed by Shimadzu UV- Spectrophotometer at 279nm.²⁰

Drug diffusion: Drug diffusion studies were carried using Franz diffusion cell. Cellophane membrane is used for studying dissolution release of gels. Diffusions studies were carried out at $37 \pm 1^\circ$ using 250 ml of phosphate buffer (pH 7.4) as the dissolution medium. Few ml of each gel sample was withdrawn periodically at every hour for 8 hrs. Samples were then analysed for drug content by using phosphate buffer as blank.²¹

Stability Study: Stability studies of the formulated gel were carried out as per ICH guidelines¹⁹, by storing the gel at different temperatures and humidity conditions, viz. $25^\circ\text{C} \pm 2^\circ\text{C}/ 60\% \pm 5\%$ RH, $30^\circ\text{C} \pm 2^\circ\text{C}/ 65\% \pm 5\%$ RH, $40^\circ\text{C} \pm 2^\circ\text{C}/ 75\% \pm 5\%$ RH for a period of three months and studied for appearance, pH, and spreadability.

Skin irritation Study: Skin irritation study was performed on guinea pigs of either sex weighing 150 - 200g. They are supplied with standard feed and required amount of water. Depilation of hair was carried out at the area of 4 cm^2 and marked on sides of guinea pig, one serving as control and the other test. Application of gel was done twice daily for seven days. The site of the skin was observed for any sensitivity and the reaction if any is graded as 0-no reaction, 1-slight patchy erythema, 2-moderate patchy erythema and 3-severe erythema with or without edema.²¹

In-vitro Antibacterial activity: Agar well diffusion method^{22, 23} was employed for performing the antibacterial assay of the optimized gel, ME₁.

The Muller Hinton Agar No.2 media along with 10^8 cfu/ml inoculum (Hi-Media) was poured into petri plate. The cup-borer was used to prepare wells in the petri plates. 100 μ l optimized gel was pipetted and then added into the well. The plates are incubated for 12hrs at 37 $^{\circ}$ C. The diameter of zone of inhibition encompassing the bacterial growth was ascertained. Pure solvent was used as control. The zone diameter given in the **Table 6** were calculated by deducting control zone diameter from test zone diameter. The procedure was carried out three times and mean values are presented with \pm SEM.

RESULTS AND DISCUSSION: The methanolic and hydroalcoholic leaf of *Manilkara hexandra* extracts were used for preparing topical gels. Six different gels were formulated (ME₁, ME₂, ME₃, HA₁, HA₂, HA₃) using polymer carbopol in different concentrations (**Table 1**). All the preparations were evaluated for various gel parameters. The methanolic gel formulations (ME₁, ME₂, ME₃) were light green in colour, homogenous and opaque in appearance (**Table 2**). The hydroalcoholic gel formulations (HA₁, HA₂, HA₃) were dark green in colour, homogenous and opaque in appearance (**Table 2**).

The pH of all the preparations was in the range of 6.8 - 7.2, compatible for the skin (**Table 2**). Spreadability parameter, when assessed, reported the fact that all the gels were easily spreadable by the application of small amount of shear with ME₁ showing the best result, 20.38gm/sec (**Table 2**). Excellent extrudability was exhibited by all the gels (**Table 2**). Viscosity of topical gel was measured using Brookfield viscometer and the values are expressed in **Table 2**. Drug content of all the gels were determined and proclaimed in **Table 2**. The *in vitro* drug release of all the formulations were impressive, and the drug release pattern was 98.2%

at the end of 8th hour for ME₁ formulation (**Table 3, Fig. 1**).

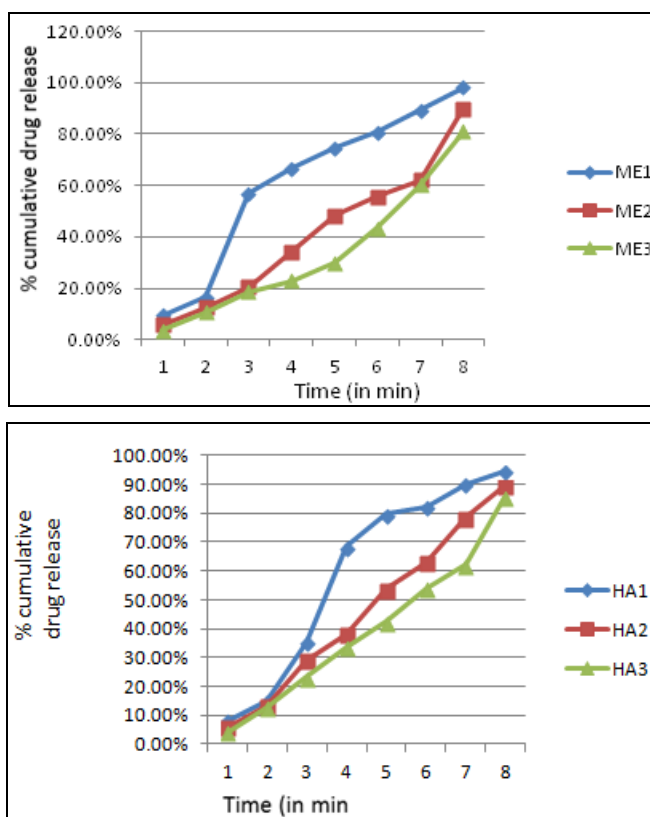


FIG. 1: % CUMULATIVE DRUG RELEASE OF METHANOLIC AND HYDROALCOHOLIC EXTRACTS

The skin irritability when tested, for seven days proffered no erythema (**Table 4**). The accelerated stability studies were conducted for all the formulations as per ICH guidelines (**Table 5**). ME₁ exhibited better stability with no change in color, appearance and negligible change in spreadability, drug content and drug release parameters, hence optimized. The optimized ME₁ topical gel was subjected to *in vitro* anti-bacterial activity against various strains and the zone of inhibition diameter (in mm) was measured. Commendable anti-bacterial activity was witnessed against the strain *Klebsiella pneumoniae* (**Table 6**).

TABLE 2: PHYSICAL EVALUATION OF ALL FORMULATIONS AT THE TIME OF GEL FORMULATION (INITIAL MONTH)

Formulation	Color	Appearance	Homogeneity	pH	Spreadability (gm.cm/sec)	Drug content (%)
ME1	Light green	Opaque	Good	7.0	20.38	98
ME2	Light green	Opaque	Good	6.8	18.91	93.8
ME3	Light green	Opaque	Good	6.9	12.35	85.71
HA1	Dark green	Translucent and opaque	Good	7.2	19.93	96
HA2	Dark green	Translucent and opaque	Good	7.1	15.62	84
HA3	Dark green	Translucent and opaque	Good	6.9	11.16	81

TABLE 3: DRUG DIFFUSION STUDIES OF ALL THE HERBAL FORMULATIONS

Time(in min)	ME ₁	ME ₂	ME ₃	HA ₁	HA ₂	HA ₃
1	9.5%	5.7%	3.8%	8.3%	5.8%	4.2%
2	16.6%	12.6%	10.6%	14.8%	13.0%	12.5%
3	56.9%	20.61%	18.6%	35.2%	29.1%	23.1%
4	66.6%	34.3%	22.8%	68.3%	37.9%	33.69%
5	74.6%	48.2%	30%	79.6%	53.6%	42.4%
6	80.7%	55.6%	43.5%	82.1%	63.2%	53.8%
7	89.2%	62.1%	60.1%	90.1%	78.5%	62.1%
8	98%	89.9%	81%	94.3%	89.3%	85.4%

TABLE 4: SKIN IRRITABILITY TESTING

Treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	0	0	0	0	0	0	0
ME1	0	0	0	0	0	0	0
ME2	0	0	0	0	0	0	0
ME3	0	0	0	0	0	0	0
HA1	0	0	0	0	0	0	0
HA2	0	0	0	0	0	0	0
HA3	0	0	0	0	0	0	0

0-no reaction, 1-slight patchy erythema, 2- moderate patchy erythema, 3-severe erythema

TABLE 5: STABILITY STUDIES OF OPTIMIZED GEL ME1 AT 3rd MONTHS

Parameters for assessment	25 ^o C±2 ^o C/60% ±5% RH	30 ^o C±2 ^o C/65% ±5% RH	40 ^o C±2 ^o C/75% ±5% RH
Appearance	Light green	Light green	Light green
Homogeneity	Good	Good	Good
Extrudability	+++	+++	+++
pH	7.1	7.0	6.8
Viscosity	8330	8210	8190
Drug content% release	97.5%	97.1%	96.9%
Drug diffusion	98.2%	98.1%	98.2%

TABLE 6: IN VITRO ANTI-BACTERIAL ACTIVITY OF OPTIMIZED GEL FORMULATION ME₁

ME ₁	Zone of inhibition (Mean±SEM)				
	Ea*	Ec*	Kp*	Pm*	Pv*
	7.5±0.9	14±0.2	19±0.5	10±0.06	4.25±0.25

*Ea-*Enterobacter aerogenes*, Ec-*Escherichia coli*, Kp- *Klebsiella pneumoniae*, Pm-*Proteus mirabilis*, Pv- *Proteus vulgaris*

CONCLUSION: Despite availability of many topical preparations, the search for better ones still continues. This contemporary research was taken up to formulate topical gels containing *Manilkara hexandra* and to optimize them. Methanolic extract of *Manilkara hexandra* (ME₁) was optimized based on the evaluation parameters and stability studies. The optimized topical gel was also found to be active against various strains of bacteria.

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CONFLICTS OF INTEREST: There are no conflicts of interest.

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