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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]

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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 anti-microbial 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

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 form 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 greesy, 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* (ME_1, ME_2, ME_3, HA_1, HA_2, HA_3) **(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 homogenously 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.

Spreadibility: Spreadibility was determined by the apparatus which consists of a wooden block, which was provided by a pulley at one end. [16] By this method spreadibility 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 spreadibility. Spreadibility was calculated using the following formula:

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

Where,
S = Spreadibility
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 ± 1° 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 guidelines [19], by storing the gel at different temperatures and humidity conditions, *viz.* 25 ^{0}C ± 2 ^{0}C/ 60% ± 5% RH, 30 ^{0}C ± 2 ^{0}C/ 65% ± 5% RH, 40 ^{0}C ± 2 ^{0}C/ 75% ± 5% RH for a period of three months and studied for appearance, pH, and spreadibility.

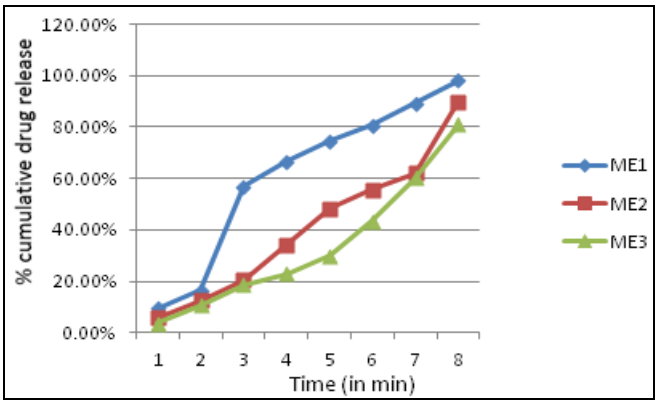
Skin irritation Study: Skin irritation study was performed on guinea pigs of either sex weighing 150 - 200g. They are supplied with standard feed and required amount of water. Depilation of hair was carried out at the area of 4 cm^2 and marked on sides of guinea pig, one serving as control and the other 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 anti-bacterial assay of the optimized gel, ME_1.

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 µl optimized gel was pipetted and then added into the well. The plates are incubated for 12hrs at 37 ^{0}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 ±SEM.

RESULTS AND DISCUSSION: The methanolic and hydroalcoholic leaf of *Manilkara hexandra* extracts were used for preparing topical gels. Six different gels were formulated (ME_1, ME_2, ME_3, HA_1, HA_2, HA_3) using polymer carbopol in different concentations **(Table 1)**. All the preparations were evaluated for various gel parameters. The methanolic gel formulations (ME_1, ME_2, ME_3) were light green in colour, homogenous and opaque in appearance **(Table 2).** The hydroalcoholic gel formulations (HA_1, HA2, HA_3) 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)**. Spreadibility 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, 20.38gcm/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)**.



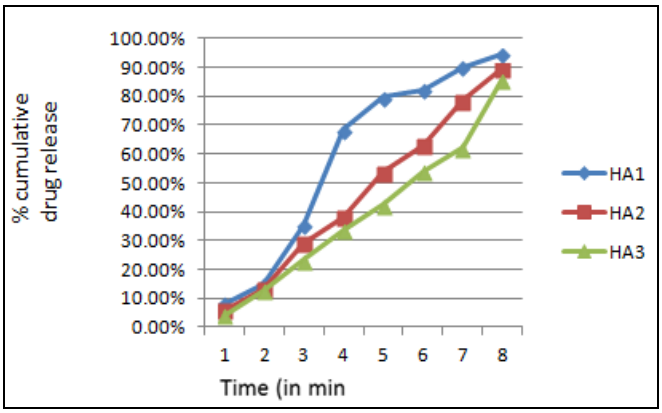


FIG. 1: % CUMULATIVE DRUG RELEASE OF METHANOLIC AND HYDROALCOHOLIC EXTRACTS

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)**. ME_1 exhibited better stability with no change in color, appearance and negligible change in spreadability, drug content and drug release parameters, hence optimized. The optimized ME_1 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	Spreadibility (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	$25^0C \pm 2^0C/60\% \pm 5\%$ RH	$30^0C \pm 2^0C/65\% \pm 5\%$ RH	$40^0C \pm 2^0C/75\% \pm 5\%$ 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 ME_1

	Zone of inhibition (Mean±SEM)				
	Ea*	Ec*	Kp*	Pm*	Pv*
ME1	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* (ME_1) 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.

REFERENCES:

1. Londonkar R L, and Hugar A L: Physicochemical, phytochemical profiling and Antimicrobial activity of *Pterocarpus marsupium*, International Journal of Pharmaceutical Sciences and Research 2017; 8: 2177-83.
2. Padmini, N, Sundaramoorthy S.D, Tripathi, H. and Hari, R: *In vitro* and *in vivo* anti-arthritic activity of combined ethanolic extracts of *Pisonia grandis* and *Cardiospermum halicacabum* in wistar rats. Journal of Applied Pharmaceutical Sciences 2016; 6(9):102-108.
3. Udupa, N: Status on herbal drugs and their future perspectives. Annals of Phytomedicine 2016; 5(1):1-3.
4. Subramoniam, A: Invited commentary: Phytomedicine for healthcare. Annals of Phytomedicine 2014 3(1):1-3.
5. Malik S.K, Choudhary R, Kumar S, Dhariwal OP and Deswal RPS: Socio-economic and horticultural potential of Khirni [*Manilkara hexandra* (Roxb.) Dubard]: a promising underutilized fruit species of India. Genetic Resources and Crop Evolution 2012; 59:1255–65.
6. Mishra. N and Pareek. A: Traditional uses, phytochemistry and pharmacology of *Mimusops hexandra* Roxb. Advances in Pharmaceutical and ethnomedicines 2014; 2 (2): 32-35

7. Parekh J, Chanda SV: Antibacterial activity of aqueous and alcoholic extracts of 34 Indian medicinal plants against some Staphylococcus species. Turkish Journal of Biology 2008; 32: 63-71.
8. Bandow JE, Brotz H, Leichert LIO, Labischinski H and Hecker M: Proteomic approach to understanding antibiotic action, Antimicrobial agents and Chemotherapy 2003; 47: 948-955.
9. Andika A, Mentari MP, Dewanti MC, Hertiani T and Pratiwi SUT: Essential oils effect in combination with antibiotics against Staphylococcus aureus ATCC 29213 Biofilm susceptibility. International Journal of Pharmaceutical Sciences and Research 2017; 8: 1606-12.
10. Colombo ML and Bosisio E: Pharmacological activities of *Chelidonium majus L* (Papaveraceae). Pharmacological Research 1996; 33: 127-34.
11. Scazzocchio F, Comets MF, Tomassini L and Palmery M. Antibacterial activity of *Hydrastis canadensis* extract and its major isolated alkaloids. Planta Medica 2001; 67: 561-63.
12. Duraipandiyan V, Ayyanar M and Ignacimuthu S: Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC Complementary and Alternative Medicine 2006; 6: 35-41.
13. Doaa A. Helal, Dalia Abd el-rhman, Sally A. Abdel-Halim, Mohamed and El-nabarawi: Formulation and evaluation of fluconazole topical gel. International Journal of Pharmacy and Pharmaceutical Sciences 2012; 4:176-183
14. Pingili D, Awasthi A and Amminbavi D. Assessment of *in vitro* antiarthritic activity of *Manilkara hexandra* (Roxb.) Dubard leaf extract. Annals of Phytomedicine. 2016; 5:152-5
15. Deepak P, Prashant B and Shamkuwar. Formulation and Evaluation of Herbal Gel Containing *Lantana Camara* leaves Extract. Asian Journal of Pharmaceutical and Clinical Research. 2013; 6:122-4.
16. Carl AB and Edward RA. Text book of clinical chemistry and molecular diagnostics, Fourth revised Edition 2001.
17. Misal G, Dixit G and Gulkari V. Formulation and evaluation of herbal gel. Indian Journal of Natural products and resources. 2012; 3:501-05.
18. Wood JH, Catacalos Gand Liberman SV: Adaptation of commercial viscometers for special applications in pharmaceutical rheology Severs extrusion rheometer. Journal of Pharm Sciences. 1963; 52:375-78.
19. ICH guidelines. Stability testing of new drug substances and products, 27th October 1993.
20. Jadhav KR, Shetye SL and Kadam VJ. Design and Evaluation of Microemulsion Based Drug Delivery System. International Journal of Advances in Pharmaceutical Sciences 2010; 1:156-66.
21. Kaur LP, Guleri TK. Topical Gel: A recent approach for novel drug discovery. Asian Journal of Biomedical and Pharmaceutical Sciences 2013; 3(17): 1-5.
22. Perez C, Paul M and Bazerque P. An antibiotic assay by the agar well diffusion method. Acta Biologica Medica 1990; 15: 113-5.
23. Nair R, Chanda S Antibacterial activity of *Punica granatum* in different solvents. Indian Journal of Pharmaceutical Sciences 2005; 67: 239-243.

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