

CODEN [USA]: IAJPBB

ISSN: 2349-7750

INDO AMERICAN JOURNAL OF PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187

Available online at: <u>http://www.iajps.com</u>

Research Article

FORMULATION EVALUATION AND DEVELOPMENT OF NATURAL HERBS CONTAINING ANTI-AGING AND ANTI-WRINKLE GEL

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Article Received: February 2023Accepted: March 2023Published: April 2023

Abstract:

The current work was done to prepare an anti-aging and anti-wrinkle Gel using an appropriate base to form a gel. The prepared gel was evaluated using various parameters and was found to be satisfied with the application on the skin to make it healthy and glowing without any side effects. Since Lavender and Rose Flower is natural anti-aging and anti-wrinkle agents, they are incorporated into the formulation which increases the efficiency of the product. Shown strong anti-wrinkle, anti-aging activity, suitable SPF for skin to protect against UV rays and provide smooth Beautifying attractive appearance to skin with lustrous and cleansing effect. Moreover, the stability study has shown no significant effect on the viscosity, homogeneity and pH of all formulations. In summary, formulation has fulfilled the cosmeceutical requirements and considered safe for skin use. Herbs are a widely distributed and widespread group of plants, excluding vegetables and otherplants consumed for macronutrients, with savory or aromatic properties that are used for flavoring and garnishing food, for medicinal purposes, or for fragrances. Culinary use typically distinguishes herbs from spices. Herbs generally refers to the leafy green or flowering parts of a plant (either fresh or dried), while spices are usually dried and produced from other parts of the plant, including seeds, bark, roots and fruits. Herbs have a variety of uses including culinary, medicinal, aromatic and in some cases, spiritual. General usage of the term "herb" differs between culinary herbs and medicinal herbs; in medicinal or spiritual use, any parts of the plant might be considered as "herbs", including leaves, roots, flowers, seeds, root bark, inner bark (and cambium), resin and pericarp

Keywords: Soxhletion, Vacuum Distillation, Flavonoids, Tannins.

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Please cite this article in press Ishwar Rubja Valvi et al, Formulation Evaluation And Development Of Natural Herbs Containing Anti-Aging And Anti-Wrinkle Gel., Indo Am. J. P. Sci, 2023; 10 (04).

INTRODUCTION:

Aging and Wrinkles are natural. as people get of age, their skin gets thinner, drier, and less elastic, and less capable to defend itself from damage. This leads to wrinkles, creases, and lines on the skin. UV light breaks the collagen and elastin fibers in the skin. These fibers form the skin's connective tissue. They are located below the surface of the skin, and they support the skin. Destroy this layer causes the skin to become weaker and less flexible. The skin starts to languish, and crinkles occurA wrinkle, also known as a rhytide, is a fold, ridge or crease in an otherwise smooth surface, such as on skin or fabric. Skin wrinkles typically appear as a result of ageing processes such as glycation,[1] habitual sleeping positions, [2] loss of body mass, sun damage, [3] or temporarily, as the result of prolonged immersion in water. Age wrinkling in the skin is promoted by habitual facial expressions, aging, sun damage, smoking, poor hydration, and various other factors.[4] In humans, it can also be prevented to some degree by avoiding excessive solar exposure and through diet (in particular through consumption of carotenoids, tocopherols and flavonoids, vitamins (A, C, D and E), essential omega-3-fatty acids, certain proteins and lactobacilli).[5]

Skin aging is a part of a natural human "aging mosaic" which becomes evident and follows different trajectories in different organs, tissues and cells with time. While the aging signs of internal organs are masked from the ambient "eyes," the skin provides first obvious marks of the passing time. Skin aging is a complex biological process influenced by combination of endogenous or intrinsic (genetics, cellular metabolism, hormone and metabolic processes) and exogenous or extrinsic (chronic light exposure, pollution, ionizing radiation, chemicals, toxins) factors.1 These factors lead together to cumulative structural and physiological alterations and progressive changes in each skin laver as well as changes in skin appearance, especially, on the sun-exposed skin areas. In contrast to thin and atrophic, finely wrinkled and dry intrinsically aged skin, premature photoaged skin typically shows a thickened epidermis, mottled discoloration, deep wrinkles, laxity, dullness and roughness.13-18 Gradual loss of skin elasticity leads to the phenomenon of sagging.19 Slowing of the epidermal turnover rate and cell cycle lengthening coincides with a slower wound healing and less effective desquamation in older adults. This fact is important when esthetic procedures are scheduled.20 On the other side, many of these features are targets to product application or procedures to accelerate the cell cycle, in the belief that a faster turnover rate will

yield improvement in skin appearance and will speed wound healing. A marked loss of fibrillin-positive structures as well as a reduced content of collagen type VII (Col-7), may contribute to wrinkles by weakening the bond between dermis and epidermis of extrinsically age skin. Sun-exposed aged skin is characterized by the solar elastosis. The sparse distribution and decrease in collagen content in photoaged skin can be due to increased collagen degradation by various matrix metalloproteinases, serine, and other proteases irrespective of the same collagen production. In older skin, collagen looks irregular and disorganized, the ratio of Col-3, to Col-1 has been shown to increase, due, significantly, to a loss of Col-1.29 The overall collagen content per unit area of the skin surface is known to decline 1%/year.Glycosaminoglycans approximately (GAGs) are among the primary dermal skin matrix constituents assisting in binding water. In photo-aged skin, GAGs may be associated with abnormal elastotic material and thus be unable to function effectively. The total hyaluronic acid (HA) level in the dermis of skin that age intrinsically remains stable; however, epidermal HA diminishes markedly. [6, 7]

Three primary structural components of the dermis, collagen, elastin and GAGs have been the subjects of the majority of anti-aging research and efforts for aesthetic-anti-aging strategies pertaining to the skin, from "anti-wrinkle creams" to various filling agents.Presentation of aging of the entire face is associated with the gravity impact, muscles action, loss of volume, diminishing and redistribution of superficial and deep fat, loss of bony skeleton support what all together lead to the face sagging, changes in shape and contour. Regardless of the fact that aging is a biological inevitable process and not a pathological condition it is correlated with various skin and body pathologies, including degenerative disorders, benign and malignant neoplasms. The 'successful aging' paradigm, focuses on health and active participation in life, counters traditional conceptualizations of aging as a time of disease and is increasingly equated with minimizing age signs on the skin, face and body. From this perspective, preventative aesthetic dermatology might supplement the request for healthy aging, treat or prevent certain cutaneous disorders, notably skin cancer, and delay skin aging combining local and systemic methods of therapy, instrumental devices and invasive procedures. The mainspring of any skin anti-aging therapy is to achieve a healthy, smooth, blemish-free, translucent and resilient skin.38 In clinical practice, "to look better" doesn't mean to "look younger." That is why it is so important to

understand patients' wishes and to orientate them to the treatment modality that will give the most satisfying results whereas knowing all available treatment techniques. The age, previous procedures or surgery, general health status, type of the skin, style of life and many other factors should be taken into consideration before choosing the strategy for the individual case. The desired therapeutic antiaging effect of the skin is continuous, step-by step process, which combines various methods of the skin bio-revitalization and rejuvenation, augmentation, restoration of each skin layer individually and in the light of many other factors—from a style of the life to the immune, genetic, emotional and health status in general. This review will emphasize the most important topical and systemic therapeutic agents and trends in the use of invasive procedures.

MATERIALS AND METHODS:

Formulation of Anti-aging and Anti-Wrinkles Gel:

Ingredients	Parts Used	Category	Qty%
Lavender	Petals	Anti-aging, Anti-Oxidant Anti-Wrinkles	5
Rose	Petals	Anti-aging, Anti-Oxidant Anti-Wrinkles	5
Carbopol 940	-	Gelling agent	3
Triethanolamine	_	Neutralizer	2
Propylene glycol	_	Moisturizer	2
Distilled water	-	Vehicle	3.85
DMDM Hydentoin	-	Preservative	0.15

Table No 1: Formulation of Anti-aging and Anti-Wrinkles Gel

EXPERIMENTAL WORK:

Extraction of Lavender using Vaccume Distillation:

Vaccume Distillation:

Vacuum distillation is distillation performed under reduced pressure, which allows the purification of compounds not readily distilled at ambient pressures or simply to save time or energy. This technique separates compounds based on differences in their boiling points. This technique is used when the boiling point of the desired compound is difficult to achieve or will cause the compound to decompose.Reduced pressures decrease the boiling point of compounds. The reduction in boiling point can be calculated using a temperature-pressure Industrial-scale vacuum distillation has several advantages. Close boiling mixtures may require many equilibrium stages to separate the key components. One tool to reduce the number of stages needed is to utilize vacuum distillation.Vacuum distillation c typically used in oil refineries have diameters ranging up to about 14 meters (46 feet), heights ranging up to about 50 meters (164 feet), and feed rates ranging up to about 25,400 cubic meters per day (160,000 barrels per day).

Vacuum distillation can improve a separation by: Prevention of product degradation or polymer formation because of reduced pressure leading to lower tower bottoms temperatures, Reduction of product degradation or polymer formation because of reduced mean residence time especially in columns using packing rather than trays.Increasing capacity, yield, and purity.Another advantage of vacuum distillation is the reduced capital cost, at the expense of slightly more operating cost.



Fig No 1- Extraction of Lavender Oil by Vaccume Distillation

Soxhlet extraction:

Soxhlet extraction has traditionally been used for a solid sample with limited solubility in a solvent in the presence of insoluble impurities. A porous thimble loaded with a solid sample is placed inside the main chamber of the Soxhlet extractor. By refluxing the solvent through the thimble using a condenser and a siphon side arm, the extraction cycle is typically repeated many times. Soxhlet extraction is a rugged, well-established technique and permits unattended extraction. However, it requires a long extraction time and the consumption of a large amount of solvent. Soxhlet extraction is a very useful tool for preparative purposes in which the analyte is

concentrated from the matrix as a whole or separated from particular interfering substances. Sample preparation of environmental samples has been developed for decades using a wide variety of techniques. Solvent extraction of solid samples, which is commonly known as solid–liquid extraction (also referred to as leaching or Lixiviation in a more correct use of the physicochemical terminology), is one of the oldest methods for solid sample pretreatment.

Procedure of Extraction of Rose oil by Soxhletion:



Preparation of gel base:



Preparation of Anti-aging and Anti-wrinkles Gel: Procedure:

The gel prepared first the Required quantity of water

was taken in a beaker then weighed quantity of propylene glycol, was added and dissolved. A weighed quantity of carbopol 940 was added little by little in a homogenizer until uniform gel was obtained. To this, extract of Natural oil was added then triturated well and at last, triethanolamine was added to adjust the pH

Evaluation of Anti-aging and Anti-wrinkle Gel:

a. pH- The pH of the gels was determined using a digital pH meter. The pH value of the Gel was 7.4 which are considered acceptable to avoid the risk of irritation on application to the skin.

b. Spreadability- The spreadability is very much important as it shows the behavior of gel that comes out from the tube. It is used to identify the extent of spreadability by the gel on the skin. A small quantity of sample was placed on a glass slide and another slide was placed above them; 100 g of weight was placed on the slide. The time taken for the gel to spread on the slide was noted and measured which was found to be 6.5 cm in 5 min. It was calculated using the following formula

S = m × 1/t S=Spreadability m=Weight placed on the slide l=Length of the glass slide t=Time taken in seconds

c. Extrudability- To determine extrudability, a closed collapsible tube containing formulation was pressed firmly at the crimped end. When the cap was removed, formulation extruded until the pressure dissipated. Weight in grams required to extrude a 0.5 cm ribbon of the formulation in 10 s was determined. The average extrusion pressure in g was reported. It was found to be 15.3 g/cm2

d. Viscosity - The viscosity of the different gel formulae was determined at 25° C using Brook field viscometer DV2T model. The gel sample (5 g) was placed in the sample holder of the viscometer and allowed to settle for 5 min, and the viscosity measured a rotating speed of 50 rpm at room temperature (25–27°C). The viscosity was found to be 1050 centipoise

e. Irritability-A small amount of gel was applied externally on the skin surface for a few minutes and checked for reactions on the skin. It was found to be non-irritant.

f. Washability- A small amount of gel was applied externally on the skin surface, and it was washed with running water. It was found to be easily washable.

Evaluation of extract:

Preliminary phytochemical screening:

- a) Flavonoids: To test solution add few drops of NaoH solution formation of dilute acid indicates presence of flavonoids.
- **b) Glycosides:** A small amount of alcoholic extract of samples is dissolved in 1ml water and then aqueous sodium hydroxide is added. Formulation of yellow colour indicates the presence of glycosides.
- c) Alkaloids (Mayer's test):1.36gm of mercuric chloride is dissolved in 60ml and 5gm of potassium iodide is dissolved in 10ml of distilled water respectively. These two solvents are mixed and dilute to 100ml using distilled water. To 1ml of acidic aqeous solution of samples few drops of reagent is added. Formation of blue or green colour indicates the presence of alkaloids.
- d) Phenols (ferric chloride test):To 1ml of alcoholic solution of sample. 2ml of distilled water followed by a few drops of 10% aqueous free chloride solution is added. Formation of blue or green colour indicates the presence of phenols.
- e) Tannins (lead acetate test):In a test tube containing about 5ml of an aqueous extract a few drops of 1% solution of lead acetate was added. Formation of a yellow or red precipitate indicate the presence of tannin.
- f) Lipids

In a test tube 5 drops of the sample was taken and a pinch of sodium hydrogen sulphate was added. Pungent odour emantes from the tube which indicates that glycerin is present which is produced by hydrolysis in fixed oil which shows the presence of lipids.

Fourier Transform Infrared spectroscopy (FT-IR)

The FT-IR spectra in no significant difference in polymer (carbopol-940), pure Natural Herbs Containing formulations

The peaks in range of 3000-3500/cm was due to alkane group (-CH3) and these were sharper in all spectrum except polymer because of the coordination of linkages. Some peaks were appeared in range of 1600 -2395/cm were due to the alkene

group(C=C) and this was sharper in polymer spectra as compare to others spectrum. This has been indicating strong bond interaction among alkene group of polymer. Whereas, peaks in range of 1020-1160/cm were due presence of phenyl group. Results of FTIR spectra of Natural Herbs Containing formulations were found to be in good agreement and suggested the stability in Natural Herbs Containing formulations with respect to carbopol-940 and penetration enhancers Natural Herbs Containing formulations

Thermal analysis:

The stability of Natural Herbs Containing formulations in Carbopol-940 was investigated by thermal analysis using TGA thermograms. The melting point of drug loaded optimized EG6 Natural Herbs Containing a formulation was revealed by the exothermic single sharp peak at -23°C. The loading temperature was 30°C. The result of thermal analysis proved the stability Natural Herbs Containing formulations at molecular level. The TGA curve of optimized Natural Herbs Containing formulations

In vitro drug release study:

The release of Natural Herbs Containing formulations was analyzed for 24 h and calculated the release amount by using regression equation for calibration curve

y = 0.0219 x + 0.1325 with regression coefficient R2 = 0.9994 at pH 7.4.

The results indicated that formulated Natural Herbs Containing formulations the highest drug release (96.69% \pm 0.01). Cumulative % drug release profile of Natural Herbs Containing formulations at pH 7.4 (n=3 \pm SD) The drug release profile of all Natural Herbs Containing formulations (EG1, EG2, EG13) at pH7.4 showed abrupt release of Natural Herbs Containing formulations due to high

Drug release kinetics:

The mode of drug release of has followed Korsemeyer-peppas model, considered as most suitable model for all formulated Natural Herbs Containing formulations at 7.4 pH due to the greatest coefficient of determination value (R2) and lowest AIC value among other models as shown in indicating that mode of drug release was not dependent on concentration of drug. The Natural

RESULT AND DISCUSSION: Evaluation Parameter: Herbs Containing formulations has shown Fickian diffusion as n < 0.45.

RSM Optimization data modeling:

The multiple linear regression analysis was utilized for creating a relationship mathematically and expressed as polynomial equation. The positive value of coefficient depicts synergistically effect while negative value shows antagonistically effect on response. The higher value of coefficient indicates that the factor has the strong impact upon response. The result of Multiple Linear Regression Analysis of response has shown % Co-efficient of variation (17.33%), F-value (3.72), R2 (0.75) and mean \pm SD (86.29 \pm 14.95).

Effect of enhancers on % drug release at Y (pH 7.4):

Significance probability P value (p>0.05) for response Y depicts that linear participation has produced non-significant effect (p< 0.05) synergistically. On the other hand, the cross product participation also produced non-significant effect (p<0.05) antagonistically while quadratic contribution A² produce significant (p>0.05) antagonistic effect while B² produce non-significant (p<0.05) effect antagonistically.

The polynomial equation is given here in terms of coded factors as:

Y = 96.75 + 38.28A + 26.35B - 20.79AB - 30.68 A2 + 4.07 B2

Optimization of Natural Herbs Containing formulations:

There was comparatively difference in drug release profile from Natural Herbs Containing formulations through cellophane membrane within 24 h time period. The results deducted from RSM data analysis, contour and 3D surface plots indicating EG6 has the maximum % drug release (96.69%) at pH 7.4 than all other Natural Herbs Containing formulations. It has revealed that Natural Herbs Containing formulations EG6 release through cellophane membrane in lesser time and depicted maximum drug release than all other formulations. EG6 Natural Herbs Containing Therefore. formulations was optimized and chosen for further investigation ex-vivo /in-vivo studies in animal/human models to confirm results.

Sr.no	Parameters	Observation	
1	Color	Colourless	
2	Odor	Aromatic	
3	Consistency	Good	
4	рН	$7.4{\pm}0.8$	
5	Viscosity	1050±0.2 centipoise	
6	Spreadability	6.5±0.6 cm	
7	Washability	Easily washable	
10	Irritability	Non-irritant	
11	Extrudability	15.3±1.2 g/cm2	

 Table No: 2 Evaluation Parameter of Anti-aging and Anti –wrinkle Gel

Evaluation of Extract:

Preliminary Phytochemical Screening:

 Table No. 3: Preliminary Phytochemical Screening

 Sr. no.
 Alkaloids
 Flavonoids
 Phenols
 glycosides
 Tannins
 Lipids

 1
 Natural Herbs
 +
 +
 +
 +
 +
 +
 +



Vaccume Distillation: Result obtained by is shown in Table below :

Weight (g)	Time (mins)
0.35	250
0.40	500
0.50	750
0.55	100
0.65	1200
0.65	1200

Table No.4 weight of oil with respect to time

The oil produced

Distillation Method is 2.45g weight of oil per 100g of dry Petals Lavender thereby producing 2.45% oil yield at 780C

Soxhletion Method:

Result obtained by Soxhlet extraction is shown in Table below:

Table No.5 weight of oil with respect to time			
Weight of oil (g)	Time (mins)		
0.4	250		
0.5	500		
0.6	750		
0.80	1000		
0.90	1200		

The amount of pure Natural Rose oil obtained by extraction method was 3.2g of essential oil per 100g of Rose Petals sample. This gave 3.02% yield of essential oil. The volume of oil was measured at every 4hr interval to determine the oil yield at varying time. As the time increases the Ethanol solvent reduces thereby leaving the oil in the mixture.

Table No.6 Result of Natural Herbs Extraction

Method of extraction	% yield
Vaccume Distillation	2.45
Soxhletion Method	3.02

Calculation of Percentage Yield of Volatile Oil: Material Balance for Vacuum Distillation Method:

- Weight of Lavender= 100g
- Quantity of hexane used= 600ml
- Quantity of Ethanol used= 200ml
- Weight of beaker= 105.26g
- Weight ethanol and essential oil= 202.7g
- The weight of oil obtained= 3.02g
- % yield = ME/MN x 100 Where,
- ME = Mass of essential oil
- ML = Mass of Lavender Petals sample
- ME = 3.02g ML = 100g

- By substituting values
- % yield = $3.02/100 \times 100 = 3.02\%$
- Therefore % yield= 3.02%

The graph below shows the plot of the weight of essential oil with respect to time for solvent extraction method



Fig No 2 Graph below shows the plot of the weight of essential oil with respect to time for Vacuum Distillation Method

Material Balance for Soxhletion Method:

- Weight of Rose Petals= 120g
- Quantity of Olive oil used= 600ml
- Quantity of Ethanol used= 140ml
- Weight of beaker= 97.86g
- Weight ethanol and essential oil= 100.41g
- The total weight= 2.45g
- % yield = ME/MR x 100 Where,
- ME = Mass of essential oil,
- MR = Mass of Rose Sample
- ME = 2.45g
- MR = 120g
- By substituting values
- % yield = 2.45/120 x 100 = 2.04%
- Therefore % yield= 2.04%

Graph of the weight (g) of essential oil to the time (mins) for extraction method





IAJPS 2023, 10 (04), 360-374

Ishwar Rubja Valvi et al

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spectra as compare to others spectrum. This has been indicating strong bond interaction among alkene group of polymer. Whereas, peaks in range of 1020-1160/cm were due presence of phenyl group. Results of FTIR spectra of Gel were found to be in good agreement and suggested the stability in Natural Herbs Containing with respect to carbopol-940 and penetration enhancers Natural Herbs.



Fig. No 4 Fourier Transform Infrared spectroscopy (FT-IR)

Thermal analysis:

The stability of Natural Herbs Containing Formulation in Carbopol-940 was investigated by thermal analysis using TGA thermograms. The melting point of drug loaded optimized EG6 Natural Herbs Containing Formulation was revealed by the exothermic single sharp peak at -23°C. The loading temperature was 30°C. The result of thermal analysis proved the stability Natural Herbs Containing Formulation at molecular level. The TGA curve of optimized Natural Herbs Containing Formulation.



Fig. No 5 Thermal analysis

In vitro drug release study:

The release of Natural Herbs Containing Formulation was analyzed for 24 h and calculated the release amount by using regression equation for calibration curve

y = 0.0219 x + 0.1325 with regression coefficient R2 = 0.9994 at pH 6.8.

The results indicated that formulated Natural Herbs Containing Formulation the highest drug release (96.69% \pm 0.01). Cumulative % drug release profile of Natural Herbs at pH 7.4 (n=3 \pm SD) The drug release profile of Natural Herbs (EG1, EG2, EG13) at pH7.4 showed abrupt release of Natural Herbs due to high

	Trial#	Coded Factor levels				
		X 1 (Extract)				
	EG1	0				
	EG2	2				
	EG3	1				
	EG4	1				
Code level		-2	-1	0	1	
X E	1 (Limonia xtract) (gm)	0.5	0.75	1.25	1.8	

Table No: 7 In vitro drug release study

Drug release kinetics:

The mode of drug release of has followed Korsemeyer-peppas model, considered as most suitable model for all formulated Natural Herbs Containing Formulation at 7.4 pH due to the greatest coefficient of determination value (R2) and lowest AIC value among other models as shown in indicating that mode of drug release was not dependent on concentration of drug. The Natural Herbs Containing Formulation has shown Fickian diffusion as n<0.45.



Fig.No 6:- Drug Release

Accelerated stability studies:

Accelerated stability studies of all prepared Natural Herbs Containing Formulation revealed that all were stable and has shown no proper significant changes in pH, consistency, % drug content and homogeneity. Only there is a slight change in color of some formulations but it did not effect on their pH, consistency, % drug content and homogeneity.



Fig. No 7 Accelerated stability studies

SUMMARY AND CONCLUSION:

From above discussion it is concluded that Natural oil Extract had anti-aging and anti-wrinkle property. From the above experimental work, the Natural oil Extract showing good activity The current work was done to prepare an anti-aging and anti-wrinkle Gel using an appropriate base to form a gel. The prepared gel was evaluated using various parameters and was found to be satisfied with the application on the skin to make it healthy and glowing without any side effects. Since Lavender and Rose Flower is natural anti-aging and anti-wrinkle agents, they are incorporated into the formulation which increases the efficiency of the product.

Shown strong anti-wrinkle, anti-aging activity, suitable SPF for skin to protect against UV rays and provide smooth Beautifying attractive appearance to skin with lustrous and cleansing effect. Moreover, the stability study has shown no significant effect on the viscosity, homogeneity and pH of all formulations. In summary, formulation has fulfilled the cosmeceutical requirements and considered safe for skin use.

Soxhletion and Vaccume distillation methods are effective and efficient means of extracting Natural Herbs Containing Gel. Extraction is the most common and most economically technique for extracting Natural oil in modern Herbal industry because of its simplicity.

Acknowledgement:

The authors are thankful to the Principal, Smt. Sharadchandrika Suresh Patil College of Pharmacy, Chopda, Maharashtra, India. Necessary facilities for research work.

Conflicts of interest:

Authors have no conflicts of interest to declare.

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