International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 12, Issue 4, 2020

Original Article

FORMULATION AND EVALUATION OF ANTI-ACNE GEL CONTAINING *MURRAYA KOEINIGII* EXTRACT

SWARNALI ROY[1], SANKHADIP BOSE[2], DHRUBAJYOTI SARKAR[3], SANJIT MANDAL[4], SIPRA SARKAR[5], SUDIP KUMAR MANDAL[6*]

[1]Department of Bioscience and Engineering, Jadavpur University, 188, Raja S. C. Mallick Road, Kolkata 700032, India, [2]Department of Pharmacognosy, Bengal School of Technology, Sugandha, Delhi Road, Chuchura, Hooghly, West Bengal 712102, India, [3]Department of Pharmaceutical Technology, NSHM Knowledge Campus, Kolkata–Group of Institutions, 124, B. L Saha Road, Kolkata 700053, India, [4]Department of Pharmaceutical Chemistry, Bengal College of Pharmaceutical Science and Research, Durgapur, West Bengal 713212, India, [5]Department of Pharmaceutical Technology, Brainware University, 398-Ramkrishnapur, Road, Barasat, Kolkata-700125, West Bengal, India, [6]Department of Pharmaceutical Chemistry, Dr. B. C. Roy College of Pharmacy and Allied Health Sciences, Durgapur 713206, West Bengal, India
Email: gotosudip79@gmail.com

Received: 25 Mar 2020, Revised and Accepted: 22 May 2020

ABSTRACT

Objective: Acne, one among the very fashionable socially distressing skin conditions created by *Propionibacterium acne* have generally been treated by antibiotics. Within the light of the growing threat of antibiotic resistance, natural plant products are applied as a safer alternative. Keeping the very fact in the background, during this research work, the formulation of gel from the extracts of *Murraya koeinigii* leaves are prepared and evaluated as an anti-acne drug.

Methods: The fresh leaf extracts were subjected to phytochemical and antimicrobial screening. Minimum Inhibitory Concentration (MIC) decided. Gel formulation of the extracts was developed and evaluated. The manufactured formulations were subjected to *In vitro* antibacterial activity against *P. acnes, S. epidermidis* and *S. aureus*. The marker compound, clindamycin, in herbal anti-acne preparation, was kept for the comparison with the zones of inhibition for antibacterial activity.

Results: Anti-acne property was explored with the help of a standard curve and by comparing diffusion profiles by taking clindamycin as a reference.

Conclusion: From the present study it can be concluded that addition of permeation enhancer in the test formulation will improve the diffusion profile and thus it was designed to add permeation enhancer.

Keywords: Acne vulgaris, *Murraya koeinigii*, *Propionibacterium acne*

© 2020 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/)
DOI: http://dx.doi.org/10.22159/ijcpr.2020v12i4.39095. Journal homepage: https://innovareacademics.in/journals/index.php/ijcpr

INTRODUCTION

Acne vulgaris is a common skin disorder which affects containing the most extensive oil glands, including the face, back and trunk [1]. *Propionibacterium acne*, an anaerobic pathogen, plays an important part in the pathogenesis of acne. It has also participated in the production of inflammatory acne by its ability to activate antioxidants and metabolize sebaceous triglycerides into fatty acids, which draw neutrophils chemotactically [2]. Acne is one of the most socially distressing skin disorders, particularly for teens, who have to cope with a disfiguring disease that erupts just when sexual maturity makes them maximum sensitive about their appearance. Moreover, permanent scarring of the skin due to severe acne leads to social distress throughout the entirely of adulthood [3]. The modification and progress of acne is due to abnormal keratinocyte proliferation and desquamation leading to ductal obstruction. Androgen driven sebum production enhances proliferation of *P. acne.* Excessive production of sebum block the pores and resulted in inflammation [4]. For many years, the use of antibiotics has shown against vulgaris. Nevertheless, the prevalence of antibiotic resistance within the dermatological setting has been increased. The progress of antibiotic resistance is multi-factorial, including the specific nature of the relationship of the bacteria to antibiotics, how the antibacterial is used, host characteristics and environmental factors [5, 6]. To avoid the problem of antibiotic resistance, researches on medicinal plants have been done as alternative treatment for the disease [7]. Leaves of *Murraya koeinigii,* a tropical tree, belonging to the family Rutaceae, used in Indian kitchen from time immemorial and possess antimicrobial [8], hypoglycemic [9] properties. Different phytoconstituents like carbazole alkaloid, mahanine have been investigated in curry leaves [10]. In our present study, alcoholic extract of leaves of *Murraya koeinigii,* was evaluated for its potential against the Acne vulgaris by *in vitro* methods.

MATERIALS AND METHODS

Plant material

The leaf of *Murraya koeinigii* (L) was collected from the Monpura forest, Adara, West Bengal on September 2015. The crude drug was identified and authenticated at the Shibpur Botanical Garden, Kolkata (Ref. No.-121BBC/BOT/2018-2019). The fresh leaves from disease free plants were washed and air-dried on sterile bottle under shade and placed in hot air oven at temp of 40 °C for 4-5 d till the weight became constant.

Microorganisms

Propionibacterium acnes (MTCC 1951) were obtained from Microbial Type Culture Collection and Gene Bank, Chandigarh, India. *Staphylocuccus epidermis*, *Staphylococcus aureus* (ATCC-29157), *Salmonelatyphi* (E-856), *Escherichia coli* (55), *Vibrio cholerae* (DN-6), *Bacillus subtilis* (US-564) were collected from the laboratory of microbiology, NSHM Knowledge Campus, Kolkata.

Drugs and chemicals

Carbopole 934, Ethanol were procured from Lobacemie, Mumbai and other chemicals like methyl paraben, propyl paraben, sodium hydroxide, triethanolamine, glycerine are of high laboratory grade and procured from Merck specialized private ltd.

Extraction procedure

The dried crude drugs were subjected to continuous hot extraction method using methanol as solvent. The solvent was selected by successive extraction method.

Phytochemical studies

Phytochemical screening of the methanol extract has been performed to determine the presence of the various phyto-constituents [11].

Preparation of gel

0.8 g of Carbopol 934 was suspended in 50 ml of distilled water with continuous stirring. The required quantities of methyl and propyl parabens were dissolved into 5 ml of distilled water by heating on a water bath. Propylene glycol 400 and Polyethylene glycol 200 were then added to the cooled solution. Further, the required quantity of methanolic extract of curry leaves as shown in table 1 was added and volume was made up to 100 ml by distilled water. After mixing all the ingredients, drop wise addition of Triethanolamine was made to the formulation for obtaining the desired consistency of the gel and to adjust the desired skin pH (6.8-7.0). The control sample was also prepared by following the same method without adding any extract.

Table 1: Formulation of gel

S. No.	Ingredient	Quantity
1	Carbopol 934	0.8 gm
2	Methyl paraben	0.2 gm
3	Propyl paraben	0.1 gm
4	Glycerine	03 ml
5	Ethanol 99.9%	20 ml
6	Double distilled water	100 ml
7	Methanolic extract of drug	0. 5 gm

Antibacterial activity test

Sample Preparations: Solutions of the plant extracts, prepared gel and marketed formulation were prepared using 100 mg of formulation in 10 ml of dimethyl sulfoxide (DMSO). Clindamycin (10 mg/ml) was used as a positive control and DMSO as a negative control [12].

Anti-acne property of hydro gel

Modified agar well diffusion method was used to detect the anti-bacterial activities of different extracts and formulations. In this method, each nutrient agar plates were planted with 0.2 ml of 24h broth culture of *S. aureus*, soybean casein digest media plates were seeded with 0.2 ml each of 24 h broth culture of *S. epidermidis* and plates of brain heart infusion media were seeded with 48 h broth culture of *P. acnes*.

The plates were dried for 1 h. In each of the plates, four equidistant wells were excavated with a sterile 8 mm borer. Into each plate, 0.5 ml of solutions of extracts, prepared poly herbal gels, Clindamycin, marketed herbal formulation and allopathic Clarithromycin gel were introduced.

The plates of *S. epidermidis* and *S. aureus* were incubated at 37 °C for 24h, and *P. acnes* were incubated for 48h. The diameter of the zones of inhibition (in mm) were measured for evaluating the antibacterial activity. The experiment was repeated four times and the mean was recorded.

Determination of minimum inhibitory concentrations (MIC)

The MIC is defined as the lowest concentration of the compound to inhibit the growth of microorganisms. The extracts were dissolved in DMSO to make a concentration of 100 mg/ml in order to determine the relative minimum inhibitory concentration values. The extracts were then diluted with DMSO to make different concentrations. To prepare different concentrations, the extracts were diluted with DMSO. Then, 100µl of these extracts were added to each cup individually. All the tests were repeated in duplicates and the mean was recorded.

Evaluation of gel

The topical formulations were assessed for colour, appearance, pH, viscosity, spreadability, stability, drug content and *in vitro* diffusion.

Physical evaluation

Physical parameters such as colour and appearance were checked visually.

Measurement of pH

The pH of various formulations was determined by using Digital pH meter. To 100 ml of distilled water, one gm of gel was dissolved and stored for 2 h. The pH of each formulation was measured in triplicate and the mean was calculated.

Viscosity

The viscosity of the prepared gels was measured with a Brookfield viscometer at a setting of 100rpm at 25 °C

Spreadability

Spreadability indicates the extent of area to which the gel readily spreads on application to skin or the affected area. The spreading value of a gel depends on its bioavailability. The spreadability is expressed in terms of time in seconds taken by two slides to slip off from the gel, placed in between the slides, under certain load. Lesser the time taken for separation of the two slides, better is the spreadability. Two sets of glass slides of standard dimensions were taken. The gel formulation was placed over one of the slides. The other slide was placed on the top of the gel so that the gel turned out a sandwich between the two slides in an area occupied by a distance of 6.0 cm along the slide. On the upper slides, a weight of 100 gm was placed so that the gel was spread uniformly into a thin layer between the two slides. The excess gel adhering to the slides was scrapped off when the weight was removed. The two slides were fixed to stand in such a position when without the slightest disturbance the weight is tied to the upper slide, it could freely slip off due to the force of the weight. To the upper slide, a weight of 20 gms was tied. The time taken for the upper slide to travel the distance of 6.0 cm was recorded. The time taken by it to separate from the lower slide under the influence of weight was also noted. The experiment was repeated in triplicate and the mean time taken was calculated.

Spread ability was calculated by using the following formula:

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

Where, S= Spreadability, M = Weight in the pan (tied to the upper slide), L = Length glass slide and T = Time (in sec.) taken to separate the slides.

Stability study

As per ICH guidelines, the stability studies were performed and the formulated gels were filled and stored in collapsible tubes at fixed condition of temperature and humidity viz. 40 °C±2 °C/75%±5% RH for a period of three months and the appearance, pH, viscosity and spreadability was studied. Results are depicted in table 9.

Drug content

The weight of each formulation (1g) was taken accurately and transferred to a 100 ml volumetric flask. 70 ml of methanol was added to it and shook. The volume was made up to 100 ml. A suitable filter paper was used to filter the contents effectively. 1 ml

filtrate was taken and diluted and the drug content (extract) was estimated using UV/Visible spectrophotometer at 250 nm.

***In vitro* diffusion study**

Preparation of standard curve of reference product

For the preparation of the standard curve of reference product, at first, a stock solution of clindamycin was prepared by dissolving 150 mg in 100 ml of distilled water. A saturated solution of 3, 6, 9, 12 and 15µg/ml concentration was prepared by withdrawing 1, 2, 3, 4 and 5 ml of stock solution respectively and dilutions was done up to 5 times. Then scanned to fix the optimum wavelength and measured the absorbance of the different concentrations of reference solution. A standard curve was obtained by plotting absorbance on Y-axis and concentration on X-axis. The slope of the standard curve was calculated.

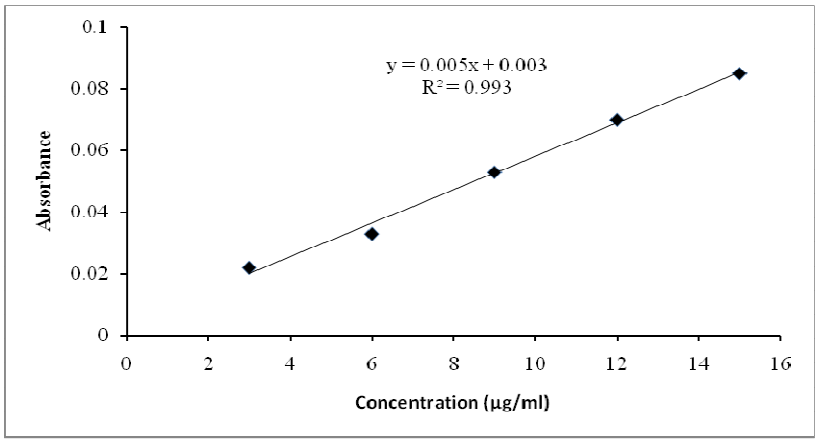

Fig. 1: Standard curve of *in vitro* diffusion study

Skin permeation study by using franz diffusion cell

In *in vitro* diffusion study of formulation and the listed reference drug was carried out in triplicate on Hanson's diffusion cell having 9 ml capacity. Permeation study of hydrogel was carried out by using mouse abdominal skin. A clean, dried receptor skin was filled with 5.5 M phosphate buffer and allowed to equilibrate at 37 °C in the heated magnetic block for 15 min. The donor compartment was then put on to the receptor compartment with dermis side of the skin facing the receptor solution containing ph 5.5 phosphate buffer. The receptor compartment was filled with buffer solution until the surface of the buffer solution touched the skin surface. The receptor solution was continuously stirred with magnetic stirrer at a temperature fixed at 37 °C±1 °C. At a periodic interval, 1 ml of sample was withdrawn and replaced with equal amount of fresh buffer simultaneously. The sample was analysed spectrophotometrically at 262 nm for sample and 213 nm for reference sample if clindamycin and absorbance was noted for further calculation, drug release profile, percentage drug release and comparisons between two release profile of sample formulation and reference standard formulation1 [13]

Diffusion profile comparison

In vitro diffusion profiles of the test formula were compared with the reference (marketed) product to provide the formulator with critical information necessary to screen formulations during product development, stability evaluation and dosage form optimization.

RESULTS AND DISCUSSION

Phytochemical analysis

The maximum product obtained from hexane extract (15.40%) in hot continuous percolation method (table 2). After performing different phytochemical tests, it has been noted that water extract showed positive test for carbohydrate and tannin, where methanol extract showed positive test for alkaloids, carbohydrate, glycosides, flavonoids and amino acids. Hexane and acetone extracts showed positive test of terpenoids, ethyl acetate extract showed positive test of glycosides, flavonoids and terpenoids, and chloroform extract showed positive test of alkaloids (table 3).

Table 2: The percentage yield of different extracts of *Murraya koeinigii*

Solvent	Weight of crude extract (gm)	% yield
Hexane	3.25	15.40
Acetone	7.50	35.54
Ethyl acetate	1.80	8.53
Chloroform	2.40	11.37
Methanol	2.90	13.74
Aqueous	3.25	15.40

Table 3: Phytochemical analysis of different extracts of *Murrayakoeinigii*

Phytochemicals	Hexane	Acetone	Ethyl acetate	Chloroform	Methanol	Aqueous
Alkaloid	-	-	-	+	+	-
Carbohydrate	-	-	-	-	+	+
Glycoside	-	-	+	-	+	-
Flavonoid	-	-	+	-	+	-
Terpenoid	+	+	+	-	+	-
Tannin	-	-	-	-	-	+
Amino acids	+	+	-	-	+	-

Antimicrobial assay

From the table 4, it is clear that concentration of the sample solution is an important parameter to control the zone of inhibition. Extract concentration must be increased to get the maximum inhibition of the probable bacteria. From the said table it has been observed that the maximum zone of inhibition was in following order-Maceration>Expression>Soxhlation>Decoction in comparison with control solution.

After performing the experimental study of anti-acne activity, table 4 showed the MIC for bacteria *S. aureus*, *S. epidermis* and *P. acne* were 50µg/ml and the effective dose of anti-acne formulation was 100 µg/ml for all type of microorganism of acne.

Table 4: Zone of inhibition of different extracts against *Propionibacterium acne*

Process of extraction		Microorganism	Conc. of extracts (gm/ml)	Zone of inhibition (cm)				Avg. zone of inhibition
Aqueous Extraction	Maceration	*Propionibacterium acne*	0.025	2.3	2.4	2.6	2.4	2.4±0.12
	Infusion		0.03	1.8	1.7	1.8	1.6	1.7±0.09
	Expression		0.01	2.2	2.0	2.4	2.3	2.2±0.17
	Decoction		0.02	1.7	1.8	1.8	1.6	1.7±0.09
Organic Extraction	Hexane		0.05	-	-	-	-	-
	Acetone		0.05	-	-	-	-	-
	Ethyl acetate		0.05	-	-	-	-	-
	Chloroform		0.05	2.0	1.9	1.8	2.0	1.9±0.09
	Methanol		0.05	-	-	-	-	-
Control	Clindamycine		0.003	2.5	2.7	2.9	2.7	2.7±0.16

Table 5: Zone of inhibition of different extracts against *S. aureus*, *S. epidermis* and *P. acne*

Extract	Conc. (µg/ml)	Zone of inhibition (mm)		
		S. aureus	*S. epidermis*	*P. acne*
Aqueous maceration extract	25	00	00	00
	50	06	12	00
	100	07	14	00
	200	08	15	00
	400	10	16	06
Clindamycin	25	02	05	00
	50	07	14	08
	100	12	16	14

Development of hydro gel

Anti-acne property of hydro gel

After performing the above anti acne activity of different formulation of hydro gel in different concentrations F1, F2 and F3 and from the table 5 and 6 it was clear that the concentration of test sample is an important parameter to control the zone of inhibition. After increasing the test sample concentration stating from 0.05% to 1%, the MIC of the stated bacteria has been increased. Also it can be concluded that, formulation F3 acquired expected zone of inhibition in respect to the reference product (Ct) of 1% clindamycin phosphate gel.

Table 6: Zone of inhibition of different formulations against *S. aureus*, *S. epidermis* and *P. acne*

S. No.	Formulation	*P. acne*			*S. aureus*			*S. epidermis*		
		Zone of inhibition (mm)		Mean (mm)	Zone of inhibition (mm)		Mean (mm)	Zone of inhibition (mm)		Mean (mm)
1.	F0	-	-	-	-	-	-	-	-	-
2.	F1 (0.05%)	09	08	8.5	12	10	11	07	07	07
3.	F2 (0.05%)	10	10	10	13	12	12.5	08	09	8.5
4.	F3 (1%)	12	13	12.5	16	15	15.5	10	10	10
5.	Reference Ct (1%)	14	14	14	17	18	17.5	15	16	15.5

Evaluation of hydro gel

Physical properties

The wavelength was found 262 nm whereas the viscosity was obtained as 1843cps at rpm 0.5 and the pH was 6.

Preparation of standard curve of reference product

Condensed tannin displayed Rf value of 0.8 in the controlled environment and tailing effect was observed, which suggesting that there was no free tannin compound left and the completion of the reaction is confirmed. Also it may be concluded that solvent system was vary in wide range in case of TLC study of condensed tannin.

Skin permeation study by using franz diffusion cell

Diffusion profile comparison

After comparing the diffusion profile of reference product (Clindamicin phosphate gel) and the test sample (formulation F2), reference product showed faster diffusion profile, it may be due to the presence of permeation enhancer in reference products which extended the permeation of the drug molecule across the different layers of skin. So from the above experiment it can be concluded that addition of permeation enhancer in the test formulation may improve the diffusion profile and thus it was decided to add permeation enhancer from here forth (table 7, 8, 9 and 10).

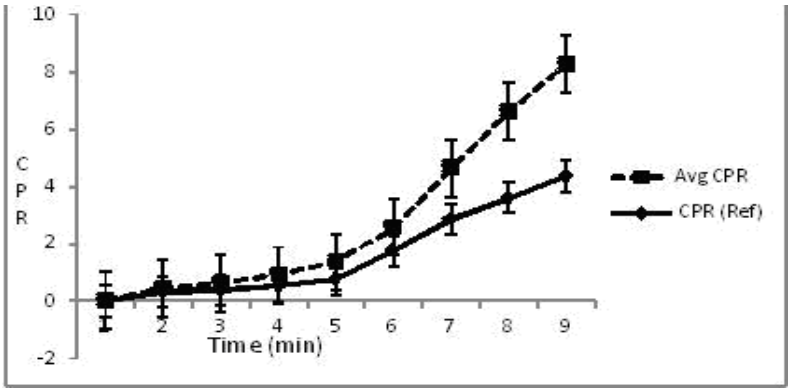

Fig. 2: Comparison between reference CPR and average CPR

Table 7: Permeability study of test formulation F1

Time (min)	Abs	Conc. (µg/ml)	In 1 ml (mg/ml)	In 9 ml	CAR in mg	CPR
30	0.002	2	0.002	0.018	0.00222	0.0074
60	0.004	4	0.004	0.036	0.038	0.126667
120	0.007	7	0.007	0.063	0.069	0.23
180	0.01	10	0.01	0.09	0.103	0.343333
240	0.017	17	0.017	0.153	0.176	0.586667
300	0.022	22	0.022	0.198	0.238	0.793333
360	0.052	52	0.052	0.468	0.53	1.766667
420	0.085	85	0.085	0.765	0.827	2.756667
480	0.11	110	0.11	0.99	1.189	3.963333

Table 8: Permeability study of test formulation F2

Time (min)	Abs	Conc. (µg/ml)	In 1 ml (mg/ml)	In 9 ml	CAR in mg	CPR
30	0.003	3	0.003	0.027	0.00222	0.0074
60	0.004	4	0.004	0.036	0.039	0.13
120	0.007	7	0.007	0.063	0.07	0.233333
180	0.015	15	0.015	0.135	0.149	0.496667
240	0.018	18	0.018	0.162	0.191	0.636667
300	0.026	26	0.026	0.234	0.281	0.936667
360	0.048	48	0.048	0.432	0.505	1.683333
420	0.098	98	0.098	0.882	0.955	3.183333
480	0.108	108	0.108	0.972	1.191	3.97

Table 9: Permeability study of test formulation F3

Time (min)	Abs	Conc. (µg/ml)	In 1 ml (mg/ml)	In 9 ml	CAR in mg	CPR
30	0.003	3	0.003	0.027	0.00222	0.0074
60	0.004	4	0.004	0.036	0.039	0.1300
120	0.006	6	0.006	0.054	0.061	0.233333
180	0.009	9	0.009	0.081	0.094	0.313333
240	0.017	17	0.017	0.153	0.175	0.583333
300	0.021	21	0.021	0.189	0.228	0.76
360	0.055	55	0.055	0.495	0.555	1.85
420	0.093	93	0.093	0.837	0.897	2.99
480	0.102	102	0.102	0.918	1.126	3.753333

Table 10: Permeability study of reference product

Time (min)	Abs	Conc. (µg/ml)	In 1 ml (mg/ml)	In 9 ml	CAR in mg	CPR
30	0.036	7.2	0.0072	0.0648	0.00222	0.0074
60	0.049	9.8	0.0098	0.0882	0.0954	0.318
120	0.054	10.8	0.0108	0.0972	0.1142	0.380667
180	0.071	14.2	0.0142	0.1278	0.1556	0.518667
240	0.103	20.6	0.0206	0.1854	0.2274	0.758
300	0.257	51.4	0.0514	0.4626	0.5252	1.750667
360	0.42	84	0.084	0.756	0.87	2.9
420	0.544	108.8	0.1088	0.9792	1.0932	3.644
480	0.56	112	0.112	1.008	1.3148	4.382667

DISCUSSION

Acne vulgaris is a general skin disease which affects common people at least once during his or her total life. Mainly in the teenage time this disease affects to the human being but many people in higher age (between 20-40 y) are also became affected by the disease. From many research, a confidence has been grown in people's mind on Herbal medications as they are safe than synthetic one. The side effects like contact allergy, local irritation, scaling, photosensitivity, itching, pruritus, redness, skin peeling, xerosis of the skin etc are the major reason to avoid synthetic drugs. The present research work done with formulation and evaluation of herbal anti-acne gels. The sample material used for the manufacturing of the formulations was from the leaf extracts of *Murraya koeinigii*. Although various topical herbal formulations for acne containing neem are available in the market, we propose to make use of *Murraya koeinigii* extract for getting better results and for the benefit of the mankind [14].

Murraya koeinigii has been marked in its previous work as a good antibacterial agent and anti-inflammatory agent [15]. The developed formulations were evaluated for them *in vitro* anti-bacterial activity against *P. acnes*, *S. epidermidis* and *S. aureus*. The Zones of inhibitions for the antibacterial activity were compared with the standard Clindamycin, marketed herbal antiacne preparation, active ingredients used in the formulation.

ACKNOWLEDGEMENT

The regards to be extended to Dr. SubhasisMaity, Director, NSHM College of Pharmaceutical Technology, NSHM Knowledge Campus, Kolkata for his enthusiastic support to conduct this work.

FUNDING

The author(s) received no specific funding for this work.

AUTHORS CONTRIBUTIONS

All of the authors contributed equally.

CONFLICT OF INTERESTS

Authors have no conflict of interest in the work and publication of this manuscript in this journal.

REFERENCES

1. Clark AK, Haas KN, Sivamani RK. Edible plants and their influence on the gut microbiome and acne. Int J Mol Sci 2017;18:1070.
2. Rocha MA, Bagatin E. Skin barrier and microbiome in acne. Arch Dermatol Res 2018;310:181–5.
3. Chilicka K, Maj J, Panaszek B. General quality of life of patients with acne vulgaris before and after performing selected cosmetological treatments. Patient Prefer Adherence 2017;11:1357–61.
4. Gollnick HPM, Dreno B. Pathophysiology and management of acne. J Eur Acad Dermatol Venereol 2015;29:1–2.
5. Adler BL, Kornmehl H, Armstrong AW. Antibiotic resistance in acne treatment. JAMA Dermato 2017;153:810–1.
6. Mandal SK. Indanyl analogs as potential antimicrobial agents. Asian J Pharm Clin Res 2018;11:278-80.
7. Nasri H, Bahmani M, Shahinfard N, Nafchi AM, Saberianpour S, Kopaei MR. Medicinal plants for the treatment of acne vulgaris: a review of recent evidences. Jundishapur J Microbiol 2015;8:11.
8. Shekar BC, Nagarajappa R, Jain R, Singh R, Suma S, Thakur R. Antimicrobial efficacy of acacia nilotica, murraya koenigii L. sprengel, eucalyptus hybrid, psidium guajava extracts and their combinations on Fusobacterium nucleatum and porphyromonas gingivalis. Indian J Dent Res 2018;29:641–5.
9. Yadav S, Vats V, Dhunnoo Y, Grover JK. Hypoglycemic and antihyperglycemic activity of Murraya koenigii leaves in diabetic rats. J Ethnopharmacol 2002;82:111–6.
10. Samanta SK, Kandimalla R, Gogoi B, Dutta KN, Choudhury P, Deb PK, *et al.* Phytochemical portfolio and anticancer activity of murraya koenigii and its primary active component, mahanine. Pharmacol Res 2018;129:227–36.
11. Rajput M, Khan RA. Phytochemical screening, acute toxicity, anxiolytic and antidepressant activities of the Nelumbo nucifera fruit. Metab Brain Dis 2017;32:743–9.
12. Balouiri M, Sadiki M, Ibnsouda SK. Methods for *in vitro* evaluating antimicrobial activity: a review. J Pharm Anal 2016;6:71–9.
13. Ng SF, Rouse J, Sanderson D, Eccleston GA. Comparative study of transmembrane diffusion and permeation of ibuprofen across synthetic membranes using franz diffusion cells. Pharmaceutics 2010;2:209–23.
14. Melnik BC, Schmitz G, Zouboulis CC. Anti-acne agents attenuate FGFR2 signal transduction in acne. J Invest Dermatol 2009;129:1868–77.
15. Rahman MM, Gray AI. A benzoisofuranone derivative and carbazole alkaloids from *Murraya koenigii* and their antimicrobial activity. Phytochemistry 2005;66:1601–6.