World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

EVALUATION OF ANTI-MICROBIAL (IN-VITRO) ACTIVITY OF ARICLEANSE CREAM

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Article Received on 30/04/2020 **Article Revised on 20/05/2020** **Article Accepted on 10/06/2020**

ABSTRACT

Ari Healthcare Pvt. Ltd. has developed Aricleanse Cream for treatment of Acne vulgaris, Hyperpigmentation and various skin diseases. The current study was conducted to evaluate anti-microbial potential of Aricleanse Cream in comparison with various formulations (F1-F8), Clindamycin and Doxycycline against *Staphylococcus aureus, Staphylococcus epidermidis* and *Propionibacterium acnes.* It was observed that F-1 to F-8 does not show anti-microbial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis.* The zone of inhibition of Aricleanse cream was 18 mm against *Staphylococcus aureus* which is less as compared to Clindamycin (26 mm) and Doxycycline (30 mm). Also, the zone of inhibition of Aricleanse cream was 12 mm against *Staphylococcus epidermidis* which is less as compared to Clindamycin (26 mm) and Doxycycline (30 mm). Formulations F1, F-2 and Aricleanse cream showed activity against *Propionobacterium acnes*. Standard drugs Clindamycin and doxycycline failed to show antimicrobial activity against *P. acnes*. It can be concluded that Aricleanse cream possesses antibacterial activity.

KEYWORDS: Aricleanse cream, Acne, *Propionibacterium acnes, Staphylococcus epidermidis.*

INTRODUCTION

Acne vulgaris is related to the pilosebaceous follicles on the skin. It is considered as an adolescent disorder characterized by formation of open & closed comedones, papules, pustules, nodules and cysts. Acne affects both males and females, although males tend to have more with onset of puberty. All over the globe, acne affects 80% of individuals between pubescence and 30 years of age.

Several factors such as disturbed hormonal (androgen) balance, excess sebum production and hyperkeratinisation are involved in pathophysiology of acne. Excessively accumulated sebum, epithelial cells and keratin obstruct the pilosebaceous follicle. This obstruction causes formation of a keratin plug and follicle swelling below skin surface resulting in acne lesion. Colonized bacteria of skin such as *Propionibacterium acnes* (*P. acnes), Staphylococcus epidermidis (S. epidermidis)* and *Staphylococcus aureus (S. aureus)* may cause severe kind of infection which leads to scarring and unpleasantness of face. Amongst these bacteria, *P. acnes* (Gram positive, non- spore forming anaerobic to aero tolerant diphtheroid bacillus) remains the most common to cause Acne vulgaris.

In modern medicine, several treatments are available for acne vulgaris but treatment must comply with type and severity of the lesions. Treatment mainly includes prolonged use of antibiotics (clindamycin, erythromycin etc.), comedolytic (retinoid, etc.) and anti-inflammatory agents. Though, these medicines are better treatment options for acne management, the side effects of these medications such as increased frequency and severity of skin dryness, scaling, erythema, burning, stinging, itching and bacterial resistance are noticeable. Furthermore, systemic antimicrobials such as clindamycin, erythromycin etc., have been associated with various short- and long-term adverse effects. Hence people are looking for alternative treatment options for acne vulgaris.

Ari Healthcare Pvt. Ltd. has conceptualized and developed Aricleanse cream for the management of acne vulgaris, hyperpigmentation and various skin disorders.

Also eight different formulae in the form of cream (F1-F8) were prepared. For the preparation of eight formulae,

we have referred four patents namely 2538/MUM/2013, 1110/CHE/2010, 1292/MUM/2011 and US2009117061. Formulations are prepared using ingredients, which are common in Aricleanse cream and that particular patent. For instance, *Manjishtha, Yashtimadhuka, Vacha* and *Lodhra* are common in patent no. 2538/MUM/2013 and Aricleanse Cream. Formulation number 1 is prepared using the same quantity of ingredients used in Aricleanse Cream.

Formulation number 2 is prepared using same ingredients but we have adjusted the quantity of ingredients to total active content of Aricleanse Cream i.e. 21%. The formulation details are mentioned in below tables. The purpose of our study was to investigate comparative antimicrobial activity of various combination (F1-F8), Aricleanse cream, Clindamycin, and Doxycycline against pathogenic bacteria responsible for the production of acne.

MATERIAL AND METHODS

The following groups were there in the study.

Table 1: Details of Group used for the study.

Name of Group	Patent Details	Batch No
Group I (Control)	NA	NA
Group II-F1	2538/MUM/2015/1	AHPL/AYCOS/1014/012
Group III-F2	2538/MUM/2015/2	AHPL/AYCOS/1014/013
Group IV-F3	1110/CHE/015/1	AHPL/AYCOS/1014/014
Group V-F4	1110/CHE/2015/2	AHPL/AYCOS/1014/015
Group VI-F5	1292/MUM/2015/1	AHPL/AYCOS/1014/016
Group VII-F6	1292/MUM/2015/2	AHPL/AYCOS/1014/017
Group VIII-F7	US2009117061/2015/1	AHPL/AYCOS/1014/018
Group IX-F8	US2009117061/2015/2	AHPL/AYCOS/1014/019
Group IX-F9	Aricleanse Cream	AC501
Group X-F10	Clindamycin	Clindac A, Alkem laboratories, Batch. No. – AA10, MFD - 10/14, EXP – 09/16
Group X-F11	Doxycycline	Doxeebest, Alto Healthcare Pvt Ltd, Batch no: MT-2005, MFG 03/2014, Exp-02/2016.

The following test products were used in the experiments:

Table 2: Details of formulations F-1 and F-2 (Reference patent no- 2538/MUM/2013).

Sr. No.	Ingredient	Botanical name	Quantity of Ingredients (%)		
			2538 (%)	AHPL/AYCOS/1014/012 (F-1)	AHPL/AYCOS/1014/013 (F-2)
1	Lodhra	*Symplocos racemosa*	2-3	3	6.3
2	Vacha	*Acorus calamus*	1-2	3	6.3
3	Yashtimadhuka	*Glycyrrhiza glabra*	3-4	2	4.2
4	Manjishtha	*Rubia cordifolia*	2-3	2	4.2
	Total			**10%**	**21%**

Table 3: Details of formulations F-3 and F-4 (Reference patent no- 1110/CHE/2010).

Sr. No.	Ingredient	Botanical name	Quantity of Ingredients (%)		
			1110 (%)	AHPL/AYCOS/1014/014 (F-3)	AHPL/AYCOS/1014/015 (F-4)
1	Lodhra	*Symplocos racemosa*	5.3	3	4.84
2	Vacha	*Acorus calamus*	5.3	3	4.84
3	Yashtimadhuka	*Glycyrrhiza glabra*	5.3	2	3.24
4	Daruharidra	*Berberis aristata*	5.3	3	4.84
	Manjishtha	*Rubia cordifolia*	5.3	2	3.24
	Total			**13%**	**21%**

Table 4: Details of formulations F-5 and F-6 (Reference patent no- 1292/MUM/2011).

Sr. No.	Ingredient	Botanical name	Quantity of Ingredients (%)		
			1292 (%)	AHPL/AYCOS/1014/016 (F-5)	AHPL/AYCOS /1014/017 (F-6)
1	Lodhra	*Symplocos racemosa*	500 mg	3	12.5
2	Shalmali	*Salmalia malabarica*	500 mg	3	12.5
	Total			**6%**	**21%**

Table 5: Details of formulations F-7 and F-8 (Reference patent no-: US2009117061).

Sr. No.	Ingredient	Botanical name	Quantity of Ingredients (%)		
			US2009 (%)	AHPL/AYCOS/1014/018 (F-7)	AHPL/AYCOS/1014/019 (F-8)
1	Dhanyaka	*Coriandrum sativum*	0.01-60	3	9
2	Yashtimadhuka	*Glycyrrhiza glabra*	0.01-60	2	6
3	Jatiphala	*Myristica fragrans*	0.01-60	2	6
	Total			**7%**	**21%**

Methods

Inoculum Preparation: Test organisms were grown in respective media to get approximately 10^6cfu organisms per ml and 100µl of this was used for the antimicrobial assay.

Method for *Staphylococcus Aureus* and *Staphylococcus Epidermidis*

Inoculation of Test Plates

The dried surface of a Mueller-Hinton agar plate was inoculated by spreading culture suspension (100µl) on agar surface.

The wells were bored in agar medium spread with the test organism, using a sterile cork borer with 8 mm inner diameter.

Aricleanse cream and other formulations were dissolved in methanol: water (50: 50) to obtain different concentrations (1% to 5%) and the known concentration (100µl) was added to the wells in triplicates.

The plates were kept in the freeze for pre-diffusion for 30 minutes then placed in an incubator set to 37°C for bacteria and 30°C for fungus for 24 hours. Clindamycin and Doxycycline were used as positive control. All experiments were performed in triplicate. The assessment of the antimicrobial activity was based on the measurement of the diameter of the zone of inhibition.

Method for *Propionibacterium Acnes*

Inoculation of Test Plates: The dried surface of a blood agar plate was inoculated by spreading 100µl culture suspension of *Propionibacterium acnes* on agar surface. The wells were bored into the surface of the inoculated agar plate and the known concentration of extracts (100µl) was added to the wells in triplicates. Plates were kept in the freeze for pre-diffusion for 30 minutes and then placed under anaerobic conditions, in an incubator set to 37°C for 48 -72 hours. Aricleanse cream (dehydrated powder of herbs used to make the cream formulation) was dissolved in methanol: water (50: 50) to obtain different concentrations (2% to 10%). The cups were bored in agar medium spread with the test organism, using a sterile cork borer with 8 mm inner diameter. These cups were filled with 100 µl test solutions and the plates were incubated at 37^0C for 48 - 72 hours under anaerobic conditions. Clindamycin and Doxycycline were used as positive control. All experiments were performed in triplicate. The assessment of the antimicrobial activity was based on the measurement of the diameter of the zone of inhibition.

Reading Plates and Interpreting Results

After incubation, each plate was examined for presence or absence of the antimicrobial activity. If activity was observed the diameters of the zones of inhibition were measured, including the diameter of the well.

RESULTS

It was observed that F-1 to F-8 does not show anti-microbial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis.* The zone of inhibition of Aricleanse cream was 18 mm against *Staphylococcus aureus* which is less as compared to Clindamycin (26 mm) and Doxycycline (30 mm). Also, the zone of inhibition of Aricleanse cream was 12 mm against *Staphylococcus epidermidis* which is less as compared to Clindamycin (26 mm) and Doxycycline (30 mm). It was also observed that only formulations F1, F-2 and Aricleanse cream showed activity against *Propionobacterium acnes*. The zone of inhibition for Aricleanse cream against *P. acnes* was 16 mm which was significantly superior to 1mm of formulation F-1 and 11mm of formulation F-2. Standard drugs Clindamycin and doxycycline failed to show antimicrobial activity against *P. acnes*.

Antibacterial Activity of Aricleanse Cream and other formulations

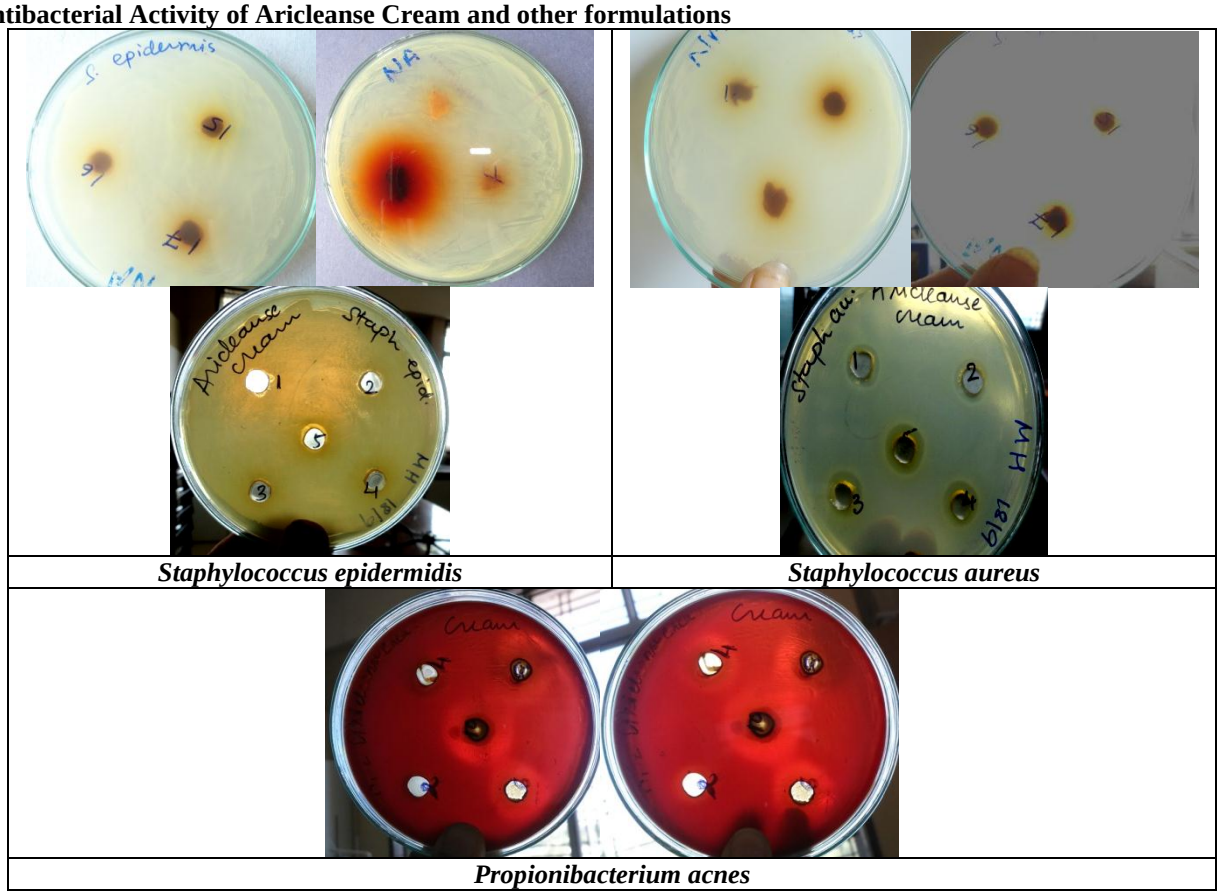

Figure 1: Antimicrobial activity of various formulations, Aricleanse cream and standard formulations (Clindamycin and doxycycline) against *Staph aureus* and *Staph epidermidis and Propionibacterium acnes.*

Table 1: Antimicrobial activity of various formulations, Aricleanse cream, Clindamycin and Doxycycline against *Staphylococcus aureus, Staphylococcus epidermidis and Propionibacterium acnes.*

Name of the test organism	Zone of inhibition (mm)										
	F-1	F-2	F-3	F-4	F-5	F-6	F-7	F-8	F9 (Aricleanse Cream)	STD Clindamycin	STD Doxycycline
Staphylococcus aureus	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	18	26	30
Staphylococcus epidermidis	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	12	26	30
Propionibacterium acnes	1	11	Nil	Nil	Nil	Nil	Nil	Nil	16	Nil	Nil

DISCUSSION

Various antibiotics like Tetracycline, Clindamycin, Erythromycin and other drugs like benzoyl peroxide are used for treatment of acne vulgaris. A major problem affecting antibiotic therapy of acne has been bacterial resistance, which has been increasing. Doxycycline can be associated with photosensitivity. Minocycline has been associated with pigment deposition in the skin, mucous membranes and teeth particularly among patients receiving long-term therapy and/or higher doses of the medication (J AM ACAD DERMATOL VOLUME 56, NUMBER 4). An increasing trend for use of alternative treatments for acne has been observed since last 10 years.

In the present study, we have investigated antimicrobial activity of Aricleanse cream in comparison with various formulations (F-1 to F-8), Clindamycin and Doxycycline against *Staphylococcus aureus, Staphylococcus epidermidis and Propionibacterium acnes*. Aricleanse Cream was conceptualized and developed by Ari Healthcare Pvt. Ltd. Our results indicate that formulations F-1 to F-8 possess no anti-microbial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis.* Aricleanse cream, Clindamycin and Doxycycline possess anti-microbial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis.* However the zone of inhibition for Aricleanse cream against *Staphylococcus aureus* and *Staphylococcus epidermidis* was found to be

less than that of Clindamycin and Doxycycline. Aricleanse cream was also effective against *Propionibacterium acnes* as evident by 16 mm zone of inhibition against it. Although formulation F1 and F2 showed antimicrobial activity against *P. acnes*, they were not as effective as Aricleanse cream. Clindamycin and Doxycycline failed to show activity against *P. acnes*. Hence it is evident that Aricleanse cream shows antibacterial activity against all the three tested organisms.

Various experiments have reported that all the ingredients of Aricleanse Cream i.e. Lodhra (*Symplocos racemosa*)[1], Vacha (*Acorus calamus*)[2], Dhanyaka (*Coriandrum sativum*)[3], Yashtimadhuka (*Glycyrrhiza glabra*)[4], Shalmali (*Salmalia malabarica*)[5], Daruharidra (*Berberis aristata*)[6], Jatiphala (*Myristica fragrans*)[7], and Manjishtha (*Rubia cordifolia*)[8] show anti-microbial activity against various pathogens. The anti-microbial activity of Aricleanse Cream against *Staphylococcus aureus*, *Staphylococcus epidermidis and Propionibacterium acnes* could be the result of synergistic action of proportionally perfect combination of various extracts present in the formulation.

CONCLUSION

It can be concluded that Aricleanse Cream possess antibacterial activity and may be used to treat acne vulgaris and other skin diseases caused due to *Staphylococcus auerus* and *Staphylococcus epidermidis and Propionibacterium acnes.*

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