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EVALUATION OF ANTI-MICROBIAL (*IN-VITRO*) ACTIVITY OF ARICLEANSE CAPSULE

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ABSTRACT

As a therapeutic agent for acne, antibiotics are typically employed to kill bacteria and as a result inflammation is reduced. However, antibiotic resistance has been increasing steadily within dermatologic setting. The development of antibiotic resistance is multifactorial. To overcome the problem of resistance, medicinal plants have been extensively studied. The potential use of traditional herbal medicines as a basis for new skin care cosmetics has been emphasized recently. It is of great importance to determine whether preparations used cosmetically in folk medicine have activities that could be useful in modern formulations. *Propionibacterium acnes* and *Staphylococcus epidermidis* are commonly detected in acne lesions, and *Staphylococcus aureus* is a common cause of skin infections. *Propionibacterium acnes* and *Staphylococcus epidermidis* are pus-forming bacteria that trigger inflammation in acne. In efforts to find new formulation of different herbal extracts for effective management of Acne vulgaris, Ari Healthcare Pvt. Ltd. has developed Aricleanse capsule containing Guduchi extract (*Tinospora cordifolia*), Manjishtha extract (*Rubia cordifolia*), Sariva extract (*Hemidesmus indicus*), Nimba extract (*Azadirachta indica*), Khadira extract *(Acacia catechu)*, Kakmachi extract (*Solanum nigrum*), and Yashtimadhuka extract (*Glycyrrhiza glabra*) which are known for their antimicrobial activities. Aricleanse capsule when tested against *Staphylococcus auerus Staphylococcus epidermidis* and *Propionibacterium acnes* was found to be active as compared to the standards Clindamycin and Doxycycline. Thus, we can conclude that Aricleanse capsule possesses antimicrobial activity and may be used to treat acne vulgaris and other skin diseases caused due to *Staphylococcus auerus and Staphylococcus epidermidis.*

KEYWORDS: Aricleanse capsule, Acne, *Propionibacterium acnes, Staphylococcus epidermidis, Staphylococcus auerus.*

1. INTRODUCTION

Acne vulgaris is related to the pilosebaceous follicle. It is considered as an adolescent disorder which is characterized by formation of open and 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. Many research studies have reported acne in 79-95% in the age group 16-18 years. In India, research studies have reported acne in 50.6% of boys and 38.13% of girls in the age group of 12-17 years.

Several factors such as disturbed hormonal (androgen) production, excess sebum production, hyperkeratinization are involved in pathophysiology of acne. Accumulation of excess 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. In these bacteria, *P. acnes* (Gram positive, non- spore forming anaerobic to aero tolerant diphtheroid bacillus) are 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 Capsule for the management of acne vulgaris, hyperpigmentation and various skin disorders.

In the current research work, we have evaluated various different formulas (prepared using different patents as reference for comparison) along with Aricleanse Capsule. Six different formulae in the form of capsule (F1-F6) were prepared. For the preparation of six formulae, we have referred three patents namely US2004/156920, US2008/206373 and 2757/DEL/2006. Formulations were prepared using ingredients, which were common in Aricleanse capsule and that particular patent. For instance, Sariva, Manjishtha, Yashtimadhuka and Nimba are common in formula used in patent no US2004/156920 and Aricleanse Capsule. Formulation F1 is prepared using the same quantity of ingredients used in Aricleanse Capsule. Formulation F2 is prepared using same ingredients but we have adjusted the quantity of ingredients to total active content of Aricleanse Capsule i.e. 480 mg. The formulation details are mentioned in below tables. The purpose of our study was to investigate comparative antimicrobial activity of different combinations (F1-F6), Aricleanse capsule, Clindamycin, and Doxycycline against pathogenic bacteria responsible for the production of acne.

2. MATERIAL AND METHODS

2.1. Borer with diameter 8 mm
2.2. Sterile Mueller Hinton plates
2.3. Sterile Potato Dextrose Agar plates
2.4. Sterile Blood Agar plates
2.5. Sterile Saline
2.6. Spreader
2.7. Alcohol
2.8. Sterile tips
2.9. Positive control (Standard Clindamycin and Doxycycline)
2.10. Test Organisms – *Staphylococcus aureus* and *Staphylococcus epidermidis, Propionibacterium acnes.*

Table No. 1: Details of group used for the study.

Name of Group	Patent Details	Batch No
Group I (Control)	NA	NA
Group II-F1	US2004/156920-1	AHPL/AYCAP/0413/007A
Group III-F2	US2004/156920-2	AHPL/AYCAP/0413/007B
Group IV-F3	US2008/206373 -1	AHPL/AYCAP/0413/007C
Group V-F4	US2008/206373 -2	AHPL/AYCAP/0413/007D
Group VI-F5	2757/DEL/2006 -1	AHPL/AYCAP/0413/007E
Group VII-F6	2757/DEL/2006 -2	AHPL/AYCAP/0413/007F
Group VIII-F7	Aricleanse Capsule	AC501
Group IX-F8	Clindamycin	Clindamycin standard drug
Group IX-F9	Doxycycline	Doxycycline standard drug

Table No. 2: Details of Formulation F1 (reference patent no. US2004/156920-1).

Sr. No.	Ingredient	Botanical name	Quantity of Ingredients (mg)	
			US2004 (%)	Batch No -AHPL/AYCAP/0413/007A
1	Sariva	*Hemidesmus indicus*	2-3	100
2	Manjishtha	*Rubia cordifolia*	1-2	70
3	Yashtimadhuka	*Glycyrrhiza glabra*	3-4	60
4	Nimba	*Azadirachta indica*	2-3	50
	Total			**280 mg**

Table No. 3: Details of Formulation F2 (reference patent no. US2004/156920-2).

Sr. No.	Ingredient	Botanical name	Quantity of Ingredients (mg)	
			US2004 (%)	Batch No -AHPL/AYCAP/0413/007B
1	Sariva	*Hemidesmus indicus*	2-3	172
2	Manjishtha	*Rubia cordifolia*	1-2	120
3	Yashtimadhuka	*Glycyrrhiza glabra*	3-4	102
4	Nimba	*Azadirachta indica*	2-3	86
	Total			**480 mg**

Table No. 4: Details of formulation F3 (reference patent no. US2008/206373 -1).

Sr. No.	Ingredient	Botanical name	Quantity of Ingredients (mg)	
			US2008 (%)	Batch No -AHPL/AYCAP/0413/007C
1	Sariva	*Hemidesmus indicus*	0.1-10	100
2	Khadira	*Acacia catechu*	0.1-10	50
	Total			**150 mg**

Table No. 5: Details of formulation F4 (reference patent no. US2008/206373 -2).

Sr. No.	Ingredient	Botanical name	Quantity of Ingredients (mg)	
			US2008 (%)	Batch No -AHPL/AYCAP/0413/007D
1	Sariva	*Hemidesmus indicus*	0.1-10	320
2	Khadira	*Acacia catechu*	0.1-10	160
	Total			**480 mg**

Table No. 6: Details of formulation F5 (reference patent no. 2757/DEL/2006 -1).

Sr. No.	Ingredient	Botanical name	Quantity of Ingredients (mg)	
			2757(mg)	Batch No -AHPL/AYCAP/0413/007E
1	Sariva	*Hemidesmus indicus*	50	100
2	Guduchi	*Tinospora cordifolia*	25	100
3	Nimba	*Azadirachta indica*	50	50
	Total			**250 mg**

Table No. 7 Details of formulation F6 (reference patent no. 2757/DEL/2006 -2)

Sr. No.	Ingredient	Botanical Name	Quantity of Ingredients (mg)	
			2757(mg)	Batch No -AHPL/AYCAP/0413/007F
1	Sariva	*Hemidesmus indicus*	50	192
2	Guduchi	*Tinospora cordifolia*	25	192
3	Nimba	*Azadirachta indica*	50	96
	Total			**480mg**

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

2.12. 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 Capsule and other combinations were dissolved in methanol: water (50:50) to obtain different concentrations (1% to 5%) and the known volume (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 for 24 hours. Clindamycin and Doxycycline were used as positive control (Standard). 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.

2.13.* Method for *Propionibacterium acnes

Inoculation of Test Plates: The dried surface of a blood agar plate was inoculated by spreading culture suspension of *Propionibacterium acnes* (100µl) on agar surface. The wells were bored into the surface of the inoculated agar plate and Aricleanse capsule and other combinations were dissolved in methanol: water (50:50) to obtain different concentrations (2% to 10%) and the known volume (100µl) was added to the wells. 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. 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°C for 48 -72 hours under anaerobic conditions. Clindamycin and Doxycycline were used as positive control (Standard). 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.

2.14. 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.

3. RESULTS

Table 8: Antimicrobial activity of various formulations, Aricleanse capsule, 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 (Aricleanse Capsule)	STD Clindamycin	STD Doxycycline
Staphylococcus auerus	16	16	Nil	Nil	Nil	Nil	19	26	30
Staphylococcus epidermidis	Nil	Nil	Nil	Nil	Nil	Nil	14	29	32
Propionibacterium acnes	Nil	15	Nil	Nil	Nil	Nil	13	Nil	Nil

It was observed that F-3 to F-6 do not have anti-microbial activity against *Staphylococcus aureus.* The zone of inhibition was 16 mm each for F-1 and F-2 against *Staphylococcus auerus*, which is less as compared to Aricleanse Capsule (19 mm), Clindamycin (26 mm) and Doxycycline (30 mm). No anti-microbial activity against *Staphylococcus epidermidis* was observed for F-1 to F-6. The zone of inhibition for Aricleanse capsule against *Staphylococcus epidermidis* was found to be 14 mm, which is significantly higher than other groups (F-1 to F -6), but less than Clindamycin (29 mm) and Doxycycline (32 mm). The zone of inhibition for Aricleanse capsule and F-2 against *P. acnes* was 13 mm and 16 mm respectively suggesting anti-microbial activity. No other formulation including standard Doxycycline and clindamycin, exhibited anti-microbial activity against *Propionibacterium acnes*.

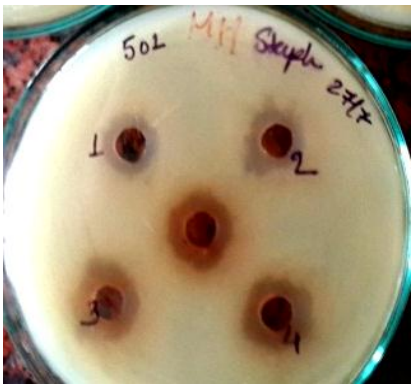

Figure No 1: Zone of inhibition for Aricleanse capsule against *Staphylococcus aureus.*

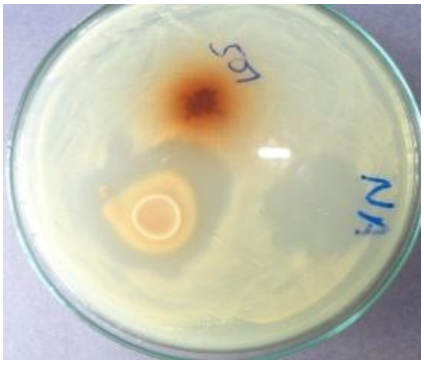

Figure No 2: Zone of inhibition *for* Aricleanse capsule against *Staphylococcus epidermidis.*

Figure No 3: Zone of inhibition for Aricleanse capsule against *Propionibacterium acnes.*

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. 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 capsule in comparison with various formulations (F-1 to F-6), Clindamycin and Doxycycline against *Staphylococcus auerus, Staphylococcus epidermidis* and *Propionibacterium acnes*. Aricleanse Capsule was conceptualized and developed by Ari Healthcare Pvt. Ltd. Results indicate that F-1 and F-2 possess anti-microbial activity against *Staphylococcus auerus.* The anti-microbial activity of Aricleanse capsule against *Staphylococcus auerus* is greater than that of F-1 and F-2, but less than that of Clindamycin and Doxycycline. No anti-microbial activity against *Staphylococcus epidermidis* was observed for F-1 to F-6. The zone of inhibition for Aricleanse capsule against *Staphylococcus epidermidis* was found to be 14, which is less than that of Clindamycin and Doxycycline. It is evident that, Aricleanse capsule and formulation F-2 exhibited anti-microbial activity against *P. acnes.* However all other formulations, Clindamycin and

Doxycycline failed to show similar activity against *P. acnes*.

Thus Aricleanse capsule possesses anti-microbial activity against *Staphylococcus auerus, Staphylococcus epidermidis and Propionibacterium acnes.*

It has been reported in various experiments that all the ingredients of Aricleanse Capsule i.e. Guduchi (*Tinospora cordifolia*)[1], Manjishtha (*Rubia cordifolia*)[2], Sariva (*Hemidesmus indicus*)[3], Nimba (*Azadirachta indica*)[4], Khadira *(Acacia catechu)*[5], Kakmachi (*Solanum nigrum*)[6], and Yashtimadhuka (*Glycyrrhiza glabra*)[7] possess anti-microbial activity against various pathogens. The anti-microbial activity of Aricleanse Capsule against *Staphylococcus auerus, Staphylococcus epidermidis and Propionibacterium acnes* could be because of the synergistic action of proportionally perfect combination of various extracts present in the formulation.

4. CONCLUSION

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

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