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Detection of *Malassezia* and Bacterial Species among Females Suffering of Dandruff in Shendi City and Antimicrobial Effect of Aloe Vera against the Isolated Microbes

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Abstract: Dandruff is a common scalp condition causing both discomfort and an undesired social image. Various studies dating from the early 1900s have investigated the condition, but an understanding of the underlying mechanisms and etiology of the condition is still in its infancy. Although dandruff isn't contagious and not serious it may be embarrassing and treatment of it difficult. This is a cross-sectional study conducted on 70 females suffering from dandruff in Shendi city in the period from July to November 2021, aimed to detect the *Malassezia* and bacterial species among females suffering from dandruff in Shendi city and the antimicrobial effect of Aloe vera against the isolated microbes. Skin swab samples were collected and cultured on both Sabroud dextrose agar containing Gentamicin and blood agar. All isolated organism was Staphylococcus species (*S. aureus*, *S. epidermids*), there have no growth of *Malassezia* species was observed on SDA. The result showed that the age group between (20-24) years was highly affected by age with *S. aureus* (74%), *and S. epidermids* (26%), and this condition was observed higher in the rural area representing (84%) of *S. aureus* and (16%) of *S. epidermids*. The oily scalp was the most common type of scalp of dandruff (76%) of *S. aureus* and (24%) of *S. epidermids*. *Staphylococcus* species were commonly found with dandruff itching, (73%) of *S. aureus* and (27%) of *S. epidermids*, and with the severity of the condition (89%) of *S. aureus* and (16%) of *S. epidermids*. Aloe vera extract showed high sensitivity against *S. aureus* (94%) and *S. epidermids* (100%). The study concluded that Staphylococcus species (*S. aureus, S. epidermids*) were the most causes of dandruff, which increased in adult age and distributed among more females from a rural areas. Staphylococcus species are found commonly with dandruff itching and the severity of dandruff. Aloe vera showed high effectiveness against *S. aureus* and *S. epidermids*.

Keywords: Dandruff, *Malassezia*, Bacterial, Aloe Vera, Antimicrobial, Females, Shendi, Sudan.

Research Paper

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INTRODUCTION

Dandruff is a chronic, recurring, and common scalp problem characterized by flaky, desquamated skin on the scalp [1]. Recently, research on the mechanism and prevention of dandruff has focused on *Malassezia*. Indeed, Malassezia alone cannot entirely explain the problem. Most scholars believe that dandruff is affected by three factors: the proliferation of *Malassezia*, abnormal sebaceous gland secretion, and individual susceptibility [2]. Most studies indicate that dandruff occurs primarily in people with strong sebaceous gland secretion and in the presence of lipophilic *Malassezia* fungi, which use sebum as a nutrient source to propagate. Therefore, most theories propose that dandruff is the result of the interaction between sebum secretion and *Malassezia* fungi. On the scalp, *Malassezia* accounts for 96% of the total number of fungi, and *M. restricta* and *M. globosaare* the dominant species. *Malassezia* represents a diverse community at the species level, and the number of species has no relation to dandruff [3]. The *Malassezia* genome possesses a large number of lipase-encoding genes compared to other fungi, enabling it to use host-derived lipids as a source of self-nutrition [4]. Dandruff is a condition, which causes small white flakes

of skin that separates and fall from the scalp. People who suffer from dandruff have overactive sebaceous glands, which make their scalp oily [5]. The scalp surface provides a distinct microenvironment to the microbes, primarily arising from the host physiological conditions, which include the sebum content, moisture, pH, and topography [6]. Microbial communities confer advantageous survival on host surfaces, such as the cutaneous sites through different regulatory processes, including biofilm formation and quorum sensing [7]. Thus, it is intended that the metabolic exchanges between the scalp surface and the microbiome typically support the growth of microbial biofilms in a symbiotic, commensal, or pathogenic form 6 comprised of 18 lipophilic species characterized as commensal yeasts, which are part of the skin mycobiota of both humans and animals [8]. And are commonly located in lipid-rich body areas, such as the scalp, face, and trunk [9]. Scalp diseases are influenced by stratum corneum integrity, immune response, and neurogenic factors. Soares *et al.,* suggested that systemic alterations of host conditions, such as Trans-epidermal Water Loss (TEWL), sebum production, and pH could alter the microbiota composition, and thereby cause dandruff. For example, sebum production is required to support the growth of Malassezia. Changes in the skin pH can create a favorable environment for the growth of Staphylococcus aureus and exert an influence on the activity of the enzymatic process of lipid metabolism in the stratum corneum, possibly contributing to the impairment of the skin barrier [10]. Dandruff (D), a mild form of seborrheic dermatitis (SD), is a skin disorder affecting 50% of the world population at some time during their lifespan regardless of gender or ethnicity [11]. Dandruff is a scalp condition that presents with the scaling of the skin. This is usually accompanied by redness on the scalp which can extend to the neck with further symptoms including loosely attached oily flakes on the skin because of pruritus [8]. These flakes can be described as separated cells of the keratinized layer with a shorter renewal period [12]. The visible symptoms of dandruff are associated with a bad social image, which causes a loss of confidence in individuals suffering from the condition [13]. It Relative abundances of two predominant bacteria, Staphylococcus and Propionibacterium, and of predominant fungi, Malassezia restricta and Malassezia globosa, behave inversely on the scalp based on disease conditions [14].

MATERIALS AND METHODS

Study Design:

This is a cross-sectional descriptive study that aimed to detect the Malassezia and bacterial species among females suffering from dandruff in Shendi city and the antimicrobial effect of Aloe vera against the isolated microbes.

Study area:

This study was conducted in Shendi city, which is a town in northern Sudan on the east bank of the River Nile 150 Km northeast of Khartoum.

Study duration: This study was done from July to November 2021.

Study population: Females suffering from dandruff.

Sample size: Seventy samples.

Data collection tools: Information from participants was collected in the performed questionnaire.

Data analysis and presentation: Data were analyzed manually by calculation of percentage.

Sample Collection:

Skin swab samples were collected by both scraping and swabbing the affected scalp using sterile combs and swabs.

Sample Culture:

Samples were cultured on both sabroud dextrose agar containing antibiotic (Gentamicin to inhibit the growth of bacteria), covered with olive oil incubated at room temperature for *Malassezia* and blood agar incubated anaerobically at 37c°, 24 hours for bacterial species, then examined it microscopically to observe colonization of *Malassezia* using potassium hydroxide and bacteria using gram stain if detected.

Culture on CLED Media:

Subculture was performed to differentiate between coagulase-negative staphylococcal species that ferment the lactose (*S. epidermids* lactose ferment).

Preparation of Aloe Vera Extraction:

To prepare the plant extract, Aloe vera was procured freshly from a greenhouse. After extraction of gel from the *Aloe vera leaf*, it was mixed and soaked with 70% ethanol at a ratio of 1 to 10 (every 100 ccs of gel was soaked with 1000 cc of ethanol), and then the mixture was placed in a shaker for 24 hours. After 24 hours, the mixture was filtered and placed in a rotary container to evaporate the solvent (ethanol). Afterward, the extract was placed in a water bath for 24 hours to form a honey-like liquid [15].

Estimation of Aloe Vera activity

After making wells by ESR tube (similar and equal to antibiotic disc) on cultured Muller Hinton agar, then 50 microliters from the extracted Aloe Vera are added to the well previously done on the medium, then incubated overnight aerobically and observed activity.

Ethical consideration

The study was reviewed and ethically approved by the scientific and the ethical committee of Shendi University and participant consent.

RESULTS

A total of seventy samples of skin scalp swabs were collected from females populations in age groups divided into 8 participants with an age group (< 20) years, 50 participants in age group (20-24) years, 12 participants in age group (25-30) years. All isolated bacteria were Staphylococcal species, *S. aureus* 52(74%), *S. epidermidis* 18 (26%). There were no *Malassezia* species isolated. High affected age group was (20-24) years with *S. aureus* 37 (74%), *and S. epidermids* 13 (26%) as shown in (Table 1). About 38 (84%) of participants from the rural area were affected with *S. aureus* while 7 (16%) were affected with *S. epidermids*; and 14 (56%) from the urban area affected with *S. aureus*, 11 (44%) affected with *S. epidermidis* as mentioned in (Table 2). About 44 (76%) of oily scalp cases were *S. aureus* and 14 (24%) were *S. epidermidis*, while 8 (67%) of the dry scalp were *S. aureus* and 4 (33%) of it were *S. epidermidis* as tabulated in (Table 3). Forty of itching suffering scalp (73%) were *S. aureus* and 15(27%) were *S. epidermidis* while 12 (80%) of not itching suffering scalp were *S. aureus* and 3 (20%) were *S. epidermidis* as shown in (Table4). Six mild cases (75%) were *S. aureus* and 2 (25%) were *S. epidermidis* while 46 (74%) of severe cases were *S. aureus* and 16 (26%) were *S. epidermidis* as mentioned in (Table 5). The antimicrobial sensitivity test of Aloe vera against isolated bacteria showed high sensitivity results for both *S. aureus* and *S. epidermidis* (94%, 100%) respectively as mentioned in (Table 6).

Table-1: The frequency of isolated bacteria according to age.

Age	***No***	***Causative agent***		***Percentage***		Total
		S. aureus	*S. epidermidis*	*S. aureus*	*S. epidermidis*	
Less than 20	8	5	3	63 %	37 %	100
20-24	50	37	13	74 %	26 %	100
25-30	12	10	2	83 %	17 %	100

Table-2: The frequency of isolated bacteria according to Geographical area.

Geographical area	***No***	***Causative agent***		***Percentage***		**Total**
		S. aureus	*S. epidermidis*	*S. aureus*	*S. epidermidis*	
Rural	45	38	7	84 %	16 %	**100**
Urban	25	14	11	56 %	44 %	**100**

Table-3: The frequency of isolated bacteria according to Type of Dandruff Scalp.

Type of dandruff scalp	***No***	***Causative agent***		***Percentage***		***Total***
		S. aureus	***S. epidermidis***	***S. aureus***	***S.epidermids***	
Oily scalp	58	44	14	76 %	24 %	**100**
Dry scalp	12	8	4	67 %	33 %	**100**

Table-4: The frequency of isolated bacteria that cause itching or not.

Suffering from itching	***No***	***Causative agent***		***Percentage***		***Total***
		S. aureus	***S. epidermidis***	***S. aureus***	***S.epidermids***	
Yes	55	40	15	73 %	27 %	**100**
No	15	12	3	80 %	20 %	**100**

Table-5: The frequency of isolated bacteria according to severity of disease.

severity of disease	***No***	***Causative agent***		***Percentage***		***Total***
		S. aureus	***S. epidermidis***	***S. aureus***	***S.epidermids***	
Mild	8	6	2	75 %	25	**100**
Severe	62	46	16	74 %	26 %	**100**

Table-6: Aloe Vera extraction susceptibility results on the isolated bacteria.

Causative agent	***No***	***Frequency***	***Sensitivity test***				
			Sensitive	***%***	***Mean of zone***	***Resist***	***%***
S. aureus	52	74 %	49	94	29	3	6%
S. epidermidis	18	26 %	18	100	28	0	0

DISCUSSION

Dandruff is the most common chronic scalp problem assigned to flaking of the skin of the scalp and its etiopathogenesis is not clearly understood. In the current study, *S. aureus* and coagulase-negative Staphylococcus were the most common bacteria isolated from the patient's scalp, which had *S. aureus* 52 (74%), *S. epidermidis* 18 (26%) and there have no *Malassezia* species isolated from dandruff scalp (0%), it may need further studies such as molecular studies to determine the actual frequencies of Malassezia. The highly affected age group was (20-24) years which were found *S. aureus* 37 (74%), *S. epidermids* 13 (26%), which may due to the activity of the sebum gland during this age. The study showed that bacteria on the scalp might vary with different geographical regions which were higher in rural areas and represents 38 (84%) of *S. aureus* and 7 (16%) of *S. epidermids,* it was probably related to hygiene or water supply. Regarding the type of skin scalp, the most common in dandruff cases was oily scalp type which had 44 (76%) of *S. aureus* and 14 (24%) of *S. epidermids.* and these findings were in agreement with the findings reported by Mayada in Tikrit University, Iraq [16]. Staphylococcus was found mostly with dandruff itching, *S. aureus* showed in 40 (73%) of cases while *S. epidermids* was found in 15 (27%) of cases suffering from itching, and this is in agreement with the study done by Lin Q *et al.*, in China [10]. also, *Staphylococcus* species were found mostly with the severity of dandruff, *S. aureus* were found in 46 (74%), and *S. epidermids* were found in 16(26%) of severe cases, and this agreement with a study done by Park T *et al.*, In china [14]. The current study showed high effectiveness of Ethanolic extracted Aloe vera gel against *S. aureus* with percent (94%) and *S. epidermids* which represent (100%) and this result was in agreement with result done by Pandey, R., Mishra, A in India [17].

CONCLUSION

All isolated organisms were *Staphylococcus* species (*S. aureus* and *S. epidermids*). Most of the dandruff cases were aged between (20 -24 yrs.), from rural residences. The oily scalp of cases was predominant. *Staphylococcus* species are found commonly with dandruff itching and with the severity of the condition. Aloe vera extract showed high effectiveness against *Staphylococcus* and can be used as a dandruff treatment.

RECOMMENDATION

a) Further studies such as molecular studies have t o be carried out to determine the actual causes of dandruff in Shendi city.
b) Clean water supply and hygiene conditions mu st be provided for people in rural areas.
c) Aloe vera showed highly effective against *Stap hylococcus* species and can be used for dandruf f treatment after further investigation and unde r medical supervision.

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