

ANTIFUNGAL ACTIVITY OF DATURA ALBA LEAVES EXTRACT AGAINST *MALASSEZIA GLOBOSA*

Afaq Ahmad

Institute of Biotechnology and Genetic Engineering, Agriculture University
Peashawar Pakistan. afaq84238@gmail.com

Mahnoor

Department of Botany Abdul wali khan university mardan kpk Pakistan.
mahnoorbtt@gmail.com

Shamshad Ali

Sarhad Institute of Allied Health Sciences Sarhad University of Science and
Information Technology Peshawar Pakistan. shamshadali9245@gmail.com

Zobia Shafi

Sarhad Institute of Allied Health Sciences Sarhad University of Science and
Information Technology Peshawar Pakistan. szubia98@gmail.com

Muhammad Saqib Khalil*

Sarhad Institute of Allied Health Sciences Sarhad University of Science and
Information Technology Peshawar Pakistan. Corresponding Author Email:
Saqib.siahs@suit.edu.pk

ABSTRACT

The main advantages of using herbal medicines are that they are relatively less dangerous compare to chemical or artificial medicines and offer profound recreational benefits. The present study aimed to evaluate the antifungal activity of *Datura alba* leaves extract against *Malassezia globosa*. *D. alba*, commonly known as Angel's trumpet, is a medicinal plant with potential antimicrobial properties. The leaves of *D. alba* were collected, dried, and prepared ethanolic and aqueous extract. The resulting extract was tested for its antifungal activity against *M. globosa*, a pathogenic fungus known to cause various infections in humans. The antifungal activity was assessed using agar well diffusion method. The extract demonstrated

significant inhibitory effects on the growth of *M. globosa*, as indicated by the zone of inhibition observed in the agar well diffusion assay. The ethanolic and aqueous were active against *M. globosa*. The ethanolic extract show maximum activity (5 mm) while aqueous extract shows moderate activity (2 mm) against *M. globosa*, for positive control Ketoconazole was used to maintain positive control during well diffusion method. The observed antifungal activity may be attributed to the presence of bioactive compounds in the extract, such as alkaloids, flavonoids, and other secondary metabolites compounds, known for their antimicrobial properties.

Keywords: Phytochemical screening, Ethanolic and Aqueous extract

INTRODUCTION

Medicinal plants have long served as a fundamental source of therapeutic agents, contributing significantly to the development of modern pharmaceuticals. A substantial proportion of contemporary drugs are derived directly or indirectly from plant sources, highlighting their importance in healthcare systems worldwide (Rates, 2001). These plants synthesize a wide range of bioactive compounds, commonly referred to as phytochemicals, which exert specific physiological effects on the human body. Such compounds, including alkaloids, flavonoids, tannins, and terpenoids, play a crucial role in disease prevention and treatment (Priya et al., 2002). The global burden of infectious diseases continues to rise, necessitating the exploration of novel and effective therapeutic agents. In this context, plant-derived natural products have gained considerable attention due to their accessibility, reduced side effects, and potential efficacy against resistant pathogens. It has been estimated that more than half of clinically used drugs originate from natural sources, underscoring the importance of continued research in this field (Bharathi, 2010).

Datura alba, a member of the family Solanaceae, is a shrub-like perennial herb widely recognized for its medicinal and ornamental significance. The genus *Datura* comprises approximately 15 species, among which *D. alba* is particularly notable due to its pharmacological properties (Fang et al., 2013). Commonly referred to as Devil's trumpet or Angel's trumpet, the plant typically grows up to 1.5 meters in height. Traditionally, it has been employed in the treatment of various ailments, including respiratory disorders, gastrointestinal disturbances, neurological conditions, and skin diseases (Mukherjee et al., 2007). The therapeutic potential of *D.*

alba is largely attributed to the presence of tropane alkaloids such as atropine, scopolamine, and hyoscyamine, which possess both medicinal and psychoactive properties. However, these compounds are also toxic at higher concentrations and may induce adverse effects such as hallucinations and delirium. In addition to its medicinal applications, *D. alba* exhibits remarkable tolerance to heavy metals, enabling it to grow in contaminated environments and contribute to phytoremediation processes (Bothe et al., 2017; Khan et al., 2019). Despite its toxicity, the plant remains a valuable candidate for pharmacological investigations due to its diverse chemical profile.

Dandruff is a common dermatological condition affecting a significant proportion of the global population, particularly individuals in the post-pubertal age group. It is primarily associated with the proliferation of *Malassezia* species, especially *Malassezia globosa*, on the scalp. The condition is characterized by excessive scaling, itching, and varying degrees of irritation or erythema, which can negatively impact an individual's quality of life (Ravichandran et al., 2004). Although dandruff is not a life-threatening condition, it poses a considerable cosmetic concern and is often accompanied by hair fall. The underlying mechanism involves disruption of the normal desquamation process, leading to the accumulation of visible flakes. Keratinocytes play a significant role in the inflammatory and immunological responses associated with dandruff formation (Piérard-Franchimont et al., 2006). Environmental factors, particularly seasonal variations, also influence the severity of the condition, with symptoms often worsening during colder months (Piérard-Franchimont et al., 1990).

The increasing prevalence of antifungal resistance and the limitations associated with existing therapeutic agents have intensified the search for alternative treatments. Plant-derived compounds offer a promising solution, as they possess inherent defense mechanisms against microbial pathogens. Numerous studies have demonstrated the antimicrobial properties of plant secondary metabolites, including their effectiveness against fungal species (Fontenelle et al., 2007). Natural products are often preferred due to their safety profile, cost-effectiveness, and reduced likelihood of resistance development compared to synthetic drugs (Ravikumar et al., 2010). For instance, certain plant extracts have shown significant antifungal activity,

indicating their potential as alternative therapeutic agents. The utilization of plant-based antimicrobials is particularly relevant in the current era, where the efficacy of conventional antifungal drugs is increasingly compromised (Khan et al., 2011).

Dandruff is closely related to seborrheic dermatitis, a more severe inflammatory condition affecting sebum-rich areas of the skin. While dandruff represents a mild, non-inflammatory form, seborrheic dermatitis involves pronounced inflammation and may extend beyond the scalp. Histological studies have revealed the presence of lymphoid cells, capillary dilation, and epidermal changes such as spongiosis and parakeratosis in affected individuals (Piérard-Franchimont et al., 2000). Although treatment responses are often rapid, recurrence is common, indicating the need for more effective and long-lasting therapeutic strategies (Arndt et al., 2007).

The etiology of dandruff is complex and multifactorial, with microbial, environmental, and host-related factors contributing to its development. Among these, the role of *Malassezia* species is widely recognized. These lipophilic yeasts colonize the scalp and proliferate under favorable conditions, leading to increased scaling and irritation. The population density of *Malassezia* has been observed to increase significantly in individuals with dandruff (Ranganathan et al., 2010). However, the exact relationship between yeast proliferation and symptom severity remains unclear. The effectiveness of antifungal treatments in reducing *Malassezia* populations supports their involvement in the condition, although recurrence following treatment discontinuation suggests additional contributing factors (Leyden, 1976).

Sebaceous gland activity is another critical factor influencing dandruff development. The condition is strongly associated with areas of high sebum production, as *Malassezia* species utilize lipids as a nutrient source. Sebum, a complex mixture of lipids, undergoes enzymatic degradation by microbial lipases, resulting in the release of free fatty acids that can trigger irritation and inflammation. Hormonal changes, stress, and environmental factors also affect sebum production, thereby influencing disease severity (Thiboutot et al., 2003; Zouboulis, 2003). Experimental studies have demonstrated that certain fatty acids, such as oleic acid, can induce dandruff-like symptoms even in the absence of

Malassezia, highlighting the interplay between microbial and host factors (Batra et al., 2005).

METHODOLOGY

Collection of plant and Preparation of Plant Extract

Fresh leaves of *D. alba* were collected randomly from the back side of Sarhad University of Information and Technology Peshawar. Leaves of *D. alba* were shade dried at temperature (32-35) for two weeks. The ethanol and aqueous extract were prepared by soaking 75g of the dry powder leaves in 600 mL (300 each) in 2 conical flasks and kept at room temperature for 48h. the extract was filtered after 48h through Whatman grade 1 filter paper. The extract was concentrated using a rotatory evaporator with the water bath set at 40 °C.

Qualitative phytochemical analysis

Test for Alkaloids

0.1g of ethanolic extract was mixed with few drops of 10% HCL for 2 min. The reaction mixture was filtered and take about 2mL in test tubes and added a few drops of Mayer's reagent to filtrate. Yellowish or white precipitate indicates presence of alkaloids (Kancherla et al. 2019).

Test for Tannin

A small quantity of *D. alba* leaves extract was mixed with water and heated on water bath and filtered. A few drops of ferric chloride were added. A dark green colour indicates the presence of tannins (Uddin et al, 2012).

Test for Flavonoids

Shinoda Test. Pieces of magnesium ribbon and HCL concentrated were mixed with ethanolic extract after few minutes and orange color showed the presence of flavonoid (Gul et al. 2017).

Alkaline Reagent Test. 2 mL of 2.0% NaOH mixture was mixed with ethanolic *D. alba* leaves extract; concentrated yellow colour was produced, which became colourless when we added 2 drops of diluted acid to mixture. This result showed the presence of flavonoids (Gul et al., 2017).

Test for Steroids

2 ml of chloroform and concentrated H₂SO₄ were added with the 5 mL of *D. alba*

aqueous extract. In the lower chloroform layer red colour appeared that indicated the presence of steroids (Gul *et al.* 2017).

Test for Terpenoids

2.0 mL of chloroform was added with the 5 mL of *D. alba* aqueous extract and evaporated on the water path and then boiled with 3 mL of H₂SO₄ concentrated. A grey colour formed which showed the entity of terpenoids (Gul *et al.* 2017).

Test for glycosides

Salkowski's Test: We added 2 mL H₂SO₄ concentrated to the whole aqueous plant crude extract. A reddish-brown colour formed which indicated the presence of steroidal aglycone part of the glycoside (Gul *et al.* 2017).

Test for Saponin

0.2 g of *D. alba* leaves extract was shaken with 5mL of distilled water and heated to boiling. Frothing (appearance of creamy mass of small bubbles) shows presence of saponins (Uddin *et al.* 2012).

Antifungal Activity of *Datura alba*

The antifungal activity of *D. alba* leaves extract was determined using agar well diffusion method. SDA plates were overlaid with olive oil and inoculated with *M. globosa* by spreading on the surface of the media. A well was made in the center of the medium and the extract (10 µg/µl) was loaded in the well. Ketoconazole (10 µg) was maintained as positive control. The plates were incubated at 32 °C for 3-4 days. The antifungal activity was assessed by measuring the diameter of the zone of inhibition (in mm) (Sakthi *et al.* 2011).

RESULTS

Qualitative phytochemical analysis

The result of the phytochemical screening of *D. alba* presented in the Table 1 show that Terpenoids, Flavonoids, and Glycosides, steroids are present in both aqueous and ethanol extract. Saponin was present in aqueous extract and not present in ethanolic extract, while Alkaloids were absent in both extracts. The color of both extracts changes to orange indicates the presence of Flavonoids. Red color is formed in both extracts show the presence of steroids and terpenoids. Dark green color in both extracts show the presence of tannin, and the aqueous extracts color change

into reddish brown which indicates presence of glycosides.

Table 1: *Qualitative analysis of ethanol and aqueous extracts of D. alba leaves*

S. No	Phytochemical tests	Aqueous Extracts	Ethanol Extracts
1	Test for Alkaloids	—	—
2	Test for Tannin	+	+
3	Test for Flavonoids	+	+
4	Test for Steroids	+	+
5	Test for Terpenoids	+	+
6	Test for Saponin	+	—
7	Test for Glycosides	+	—

Antifungal Activity

In this current study activity of *D. alba* was seen against *M. globosa*. The ethanolic and aqueous extracts *D. alba* both were active against *M. globosa*. The ethanolic extract show maximum activity about (5 mm) against *M. globosa* while the aqueous extract shows moderate activity about (2 mm) against *M. globosa*. The antifungal drug use in this current study was ketoconazole show maximum activity about (15 mm) against *M. globosa*. The following expression was used for calculation of zone of inhibition of fungus and antifungal drug for both extracts of *D. alba* %inhibition = $\frac{\text{Zone of inhibition of total sample/control} \times 100}{\text{Zone of inhibition of total sample/control}}$

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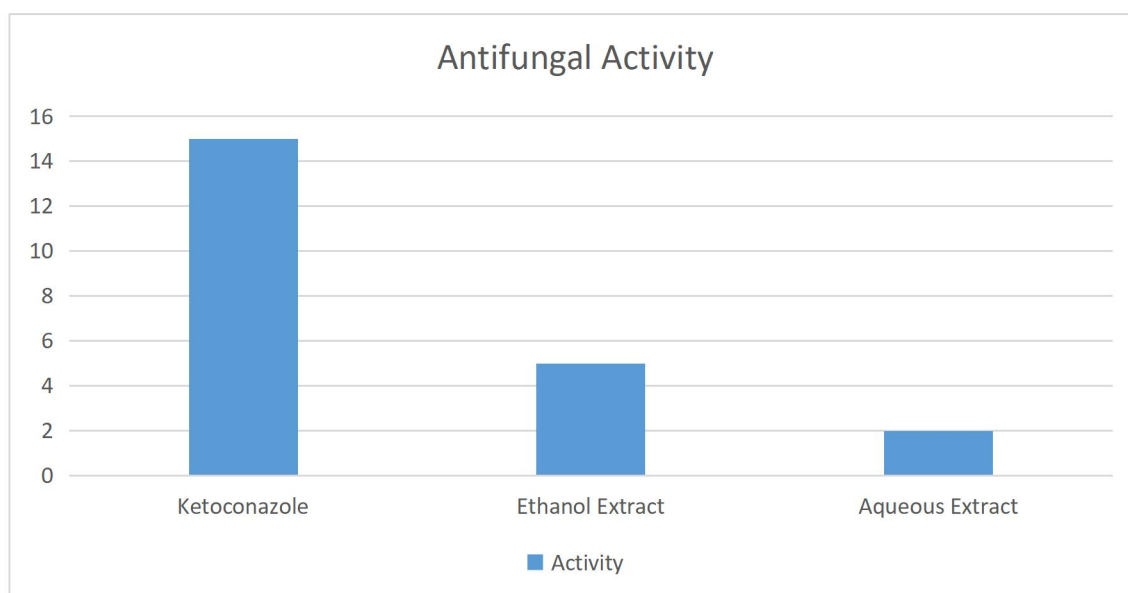


Fig 1: Antifungal Activity of *Datura Alba* leaves extracts

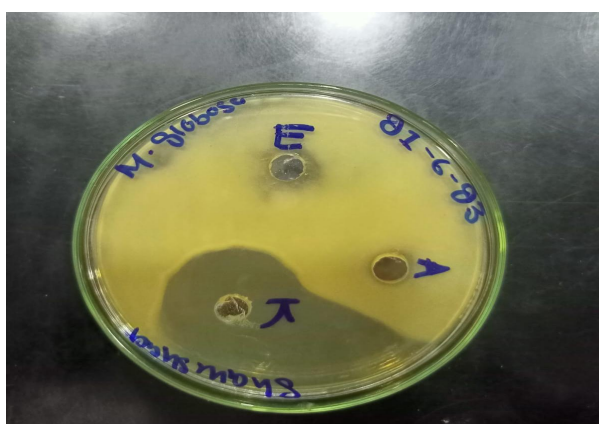
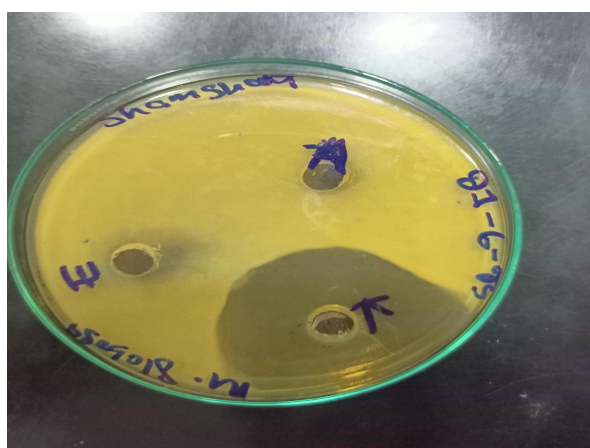


Fig 2 Antifungal activity of ethanolic and Aqueous extracts *D. alba* against *M. globosa*

DISCUSSIONS

D. alba has been used in some traditional systems of medicine for various purposes and several studies exhibit their vital in respiratory conditions, pain relief, anti-inflammatory and anti-ulcer activities. These biological activities are related with phytochemical composition of *D. alba* leaves. The ethanolic extract gave more yield than the aqueous extract. This probably infers that ethanol had better solubility, extracting and quantitative abilities of the active components from *D. alba* leaves than water. Earlier study by Sepehri et al., (2016) reported that ethanolic extract had better antifungal activity than aqueous of plant extracts.

Waliullah and sidra (2019) reported the presence of flavonoids, tannins, and phenols are in all the four parts (leaf, stem, seed, and root) of *D. alba*. Rauf and Samina (2012) investigate the phytochemical constitutions of five leaves extracts of *D. alba* (n-hexane, Chloroform, Ethyl acetate, methanol, Ethanol and water) and their results support the results obtained in the present study.

This study demonstrates that both leaves extracts had antifungal activity against *M. globosa*. However, ethanolic extracts provide better antifungal activity than aqueous extract. Forgerman (1996) reported that Dandruff is caused by the commensal lipophilic yeast and hence cannot be completely eliminated but can only be managed. Recent years for the treatment of dandruff, a variety of the synthetic chemicals with anti-Malassezia activity such as Ketoconazole, Zinc pyrithione, Octopirox, Climbazole. In this current investigation *M. globosa* show selective susceptibility against antifungal drug Ketoconazole within the range of zone of inhibition between 14-15 mm so their results support the results obtained in the current study

Manju et al. (2012) reported that *Datura* extracts show moderate activity against *M. globosa*. In current investigation ethanolic extract of *D. alba* shows maximum activity within range of zone of inhibition between 4-5 mm while the aqueous extract of *D. alba* shows minimum active against *M. globosa* within the range of zone of inhibition between 1.5-2 mm so their results support the results obtained in the present study.

CONCLUSION

According to the result of current study *D. alba* is a good source of various of phytochemicals, including alkaloids, flavonoids, tannin, steroids, terpenoids, glycosides, and saponins. The current study demonstrated the antifungal activity of *D. alba* against *M. globosa*. The ethanolic extract of *D. alba* show maximum activity about (5 mm) against *M. globosa* while the aqueous extract shows moderate activity about (2 mm) against *M. globosa*.

REFERENCES

- Arndt, K. A., & Hsu, J. T. (2007). *Manual of dermatologic therapeutics*. Lippincott Williams & Wilkins.
- Batra, R., Boekhout, T., Guého, E., Cabanes, F. J., Dawson Jr, T. L., & Gupta, A. K. (2005). *Malassezia Baillon*, emerging clinical yeasts. *FEMS yeast research*, 5(12), 1101-1113.
- Bharathi, B., Sharmiladevi, R., & Swamidoss, G. D. (2010). Studies on antibacterial activity and phytochemical analysis of *Datura metel* L. against bacterial pathogens associated with HIV. *Advanced Biotech*, 10(03), 1.
- Bothe, H., & Słomka, A. (2017). Divergent biology of facultative heavy metal plants. *Journal of Plant Physiology*, 219, 45-61.
- Fang, S. T., Liu, X., Kong, N. N., Liu, S. J., & Xia, C. H. (2013). Two new withanolides from the halophyte *Datura stramonium* L. *Natural Product Research*, 27(21), 1965-1970.
- Fontenelle, R. O. S., Morais, S. M., Brito, E. H. S., Kerntopf, M. R., Brilhante, R. S. N., Cordeiro, R. A., ... & Rocha, M. F. G. (2007). Chemical composition, toxicological aspects and antifungal activity of essential oil from *Lippia sidoides* Cham. *Journal of Antimicrobial Chemotherapy*, 59(5), 934-940.
- Forgeman, L. (1996). The etiology of dandruff and its treatment. *Journal of the American Academy of Dermatology*, 35(6), 101-105.
- Gul, R., Jan, S. U., Faridullah, S., Sherani, S., & Jahan, N. (2017). Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from *Ephedra intermedia* indigenous to Balochistan. *The Scientific World Journal*, 2017.

- Kancherla, N., Dhakshinamoothi, A., Chitra, K., & Komaram, R. B. (2019). Preliminary analysis of phytoconstituents and evaluation of anthelmintic property of *Cayratia auriculata* (in vitro). *Maedica*, 14(4), 350.
- Khan, J. A., & Hane, S. (2011). Antibacterial properties of *Punica granatum* peels. *Int J Apply Biol Pharmaceut Technol*, 2(3), 23-7.
- Khan, W., Subhan, S., Shams, D. F., Afridi, S. G., Ullah, R., Shahat, A. A., & Alqahtani, A. S. (2019). Antioxidant potential, phytochemicals composition, and metal contents of *Datura alba*. *BioMed research international*, 2019.
- Leyden, J. J., McGinley, K. J., & Kligman, A. M. (1976). Role of microorganisms in dandruff. *Archives of Dermatology*, 112(3), 333-338.
- Manju, K., Sharma, R., & Singh, S. (2012). Antifungal activity of *Datura* extracts against *Malassezia* species. *International Journal of Pharmaceutical Research and Development*, 4(7), 123-128.
- Mukherjee, P. K., Rai, S., Kumar, V., Mukherjee, K., Hylands, P. J., & Hider, R. C. (2007). Plants of Indian origin in drug discovery. *Expert Opinion on Drug Discovery*, 2(5), 633-657.
- Pierard-Franchimont, C., Hermanns, J. F., Degre, H., & Pierard, G. E. (2000). From axioms to new insights into dandruff. *Dermatology*, 200(2), 93-98.
- Pierard-Franchimont, C., Pierard, G. E., & Kligman, A. (1990). Seasonal modulation of sebum excretion. *Dermatology*, 181(1), 21-22.
- Pierard-Franchimont, C., Xhaufaire-Uhoda, E., & Pierard, G. E. (2006). Revisiting dandruff. *International journal of cosmetic science*, 28(5), 311-318.
- Priya, K. S., Gnanamani, A., Radhakrishnan, N. O., & Babu, M. (2002). Healing potential of *Datura alba* on burn wounds in albino rats. *Journal of ethnopharmacology*, 83(3), 193-199.
- Ranganathan, S., & Mukhopadhyay, T. (2010). Dandruff: the most commercially exploited skin disease. *Indian journal of dermatology*, 55(2), 130.
- Rates, S. M. K. (2001). Plants as source of drugs. *Toxicon*, 39(5), 603-613.
- Rauf, A., & Samina, S. (2012). Phytochemical analysis and antioxidant potential of different solvent extracts of *Desmostachya bipinnata*. *African Journal of Pharmacy and Pharmacology*, 6(22), 1600-1605.

- Ravichandran, G., Bharadwaj, V. S., & Kolhapure, S. A. (2004). Evaluation of the clinical efficacy and safety of “Anti-Dandruff Shampoo” in the treatment of dandruff. *The Antiseptic*, 201(1), 5-8.
- Ravikumar, S., Selvan, G. P., & Gracelin, A. A. (2010). Antimicrobial activity of medicinal plants along Kanyakumari coast, Tamil Nadu, India. *Afr. J. Basic Appl. Sci*, 2(5-6), 153-157.
- Sakthi, S. S., Geetha, M., & Saranraj, P. (2011). Pharmacological screening of *Datura metel* and *Acalypha indica* for its antifungal activity against pathogenic fungi. *International journal of pharmaceutical science and health care*, 2(1), 15-30.
- Sepehri, Z., Javadian, F., Khammari, D., & Hassanshahian, M. (2016). Antifungal effects of the aqueous and ethanolic leaf extracts of *Echinophora platyloba* and *Rosmarinus officinalis*. *Current medical mycology*, 2(1), 30.
- Thiboutot, D., Gilliland, K., Cong, Z., Jabara, S., McAllister, J. M., Sivarajah, A., & Clawson, G. (2003). Human skin is a steroidogenic tissue: steroidogenic enzymes and cofactors are expressed in epidermis, normal sebocytes, and an immortalized sebocyte cell line (SEB-1). *Journal of investigative dermatology*, 120(6), 905-914.
- Uddin, G., Rauf, A., & Akhtar, S. (2012). Studies on chemical constituents, phytochemical profile and pharmacological action of *Datura alba*. *Middle-East Journal of Medicinal Plants Research*, 1(1), 14-18.
- Waliullah, H., & Sidra, S. (2019). Phytochemical screening and evaluation of bioactive compounds in different parts of *Desmostachya bipinnata* (L.) Stapf. *Journal of Medicinal Plants Research*, 13(5), 85-92.
- Zouboulis, C. C. (2003). Sebaceous gland in human skin--the fantastic future of a skin appendage. *The Journal of investigative dermatology*, 120(6), xiv-xv.