International Journal of Research and Publication
Issue 5:Vol. 2 (May, 2023)

THERAPEUTIC POTENTIAL OF CHROMOLAENA ODORATA LEAVES EXTRACT AGAINST KERATINOPHILIC FUNGAL INFECTION

Amakor Chilota Adaobi and Obi Chisom Perpetua

Department of Science Laboratory and Technology (Microbiology option)

Federal Polytechnic Oko.Anambra State.Nigeria.

08164549689, 08065875124

amakorchilota@gmail.com, obipchisom@gmail.com

Abstract

The objective of this research was to evaluate the therapeutic efficacy of an antikeratinophilic cream formulated using an extract derived from *Chromolena odorata* leaves (COL). The collection of garden soil samples was conducted in a random manner, and subsequent analysis included the screening for the presence of keratinophilic fungus using microbiological methods that were deemed acceptable for this purpose. The evaluation of the pathogenic potentials of the isolates was conducted utilizing an in vivo methodology. An in vivo approach was used, using albino Wistar rats, to assess the therapeutic efficacy of the produced cream against keratinophilic fungal infections.The data obtained from the research were subjected to statistical analysis using Analysis of Variance (ANOVA), Chi square (X2), and student 't' test at a confidence level of 95%. The findings of the study revealed that the soil samples included *Microscoporum* species (MS), *Trichophyton* species (TRS), and Trichophyton species (TS).The isolates demonstrated statistically significant ($P < 0.05$). Pathological lesions, with the highest incidence seen in the rats infected with TS. The cream that was made had a smooth and thick consistency, as shown by its viscosity of 46.70 mPa.S. It also possessed specific gravity, refractive index, and pH values of 0.904, 1.460, and 7.30, respectively. Furthermore, the cream demonstrated absence of microbial load, as evidenced by zero counts in all examined parameters. The cream exhibited notable therapeutic efficacy against the infected tissues, with the highest level of resolution seen during a time-frame of 42 to 56 days. This result was statistically significant ($P < 0.05$), particularly in relation to the infection induced by MS. The research findings indicate that the experimental rats had different pathological lesions when exposed to MS, TRS, and TS. However, these lesions were effectively cured utilizing a cream formulated from COL. Hence, the findings of this investigation propose that the formulated cream has potential as a viable treatment option for treating topical infections induced by keratinophilic fungus.

Keywords: Keratinophilic,*Chromolena odorata*,Pathological,Therapeutic,Fungi.

INTRODUCTION

Keratinophilic fungi are a category of fungi that have the ability to breakdown keratin, a protein present in the skin, hair, and nails of both humans and animals. The aforementioned fungus have the potential to induce a range of cutaneous illnesses, including ringworm, white piedra, and favus, which have a significant impact on a substantial global population (Sahni *et al.,* 2019). The clinical manifestations of these infections include pruritus, desquamation, inflammation, alopecia, and onychodystrophy . In addition, Gupta *et al*. (2017) have shown that these conditions may also result in bacterial infections or systemic problems. Certain fungi with a preference for keratin, such as *Microscoporum. gypseum* and *Trichophyton. mentagrophytes*, have the ability to be transferred from animals to humans (Mishra *et al.*, 2018).

The standard therapeutic approach for addressing these infections often include the administration of antifungal medications, including azoles, allylamines, and griseofulvin. Nevertheless, it is

important to acknowledge that these pharmaceutical agents possess some limitations, including but not limited to their substantial financial burden, limited capacity to reach target tissues, development of resistance, occurrence of unfavorable reactions, and potential interactions with other medications (Gupta et *al.*, 2017; Sahni *et al.*, 2019). Hence, there is a pressing need for the development of safe, efficacious, and cost-effective alternative medicines to address the management of these infections.

Medicinal plants has the potential to serve as a viable reservoir for alternative therapeutic interventions. Throughout millennia, several traditional systems of medicine have used them for the therapeutic management of a wide range of ailments. *C. odorata* has been identified as a medicinal plant with antifungal properties against keratinophilic fungi, as reported by Elekofehinti *et al*. (2021). The plant in question is a perennial shrub classified under the *Asteraceae* family. The species has a vast distribution in tropical and subtropical areas of Africa, Asia, and America. This plant species is often referred to as Siam weed, Christmas bush, or *Eupatorium* (Aziz *et al*., 2020).

C. odorata has been used in traditional medicine to address a range of ailments, including wounds, ulcers, burns, malaria, diarrhea, dysentery, diabetes, and inflammation (Aziz *et al.*, 2020; Elekofehinti *et al.*, 2021). The foliage of C. odorata harbors several phytochemical compounds, including flavonoids, phenolics, terpenoids, alkaloids, and steroids. The pharmacological actions of this substance may be attributed to the presence of phytochemicals, as shown by previous studies (Aziz et al., 2020; Elekofehinti *et al*., 2021). Multiple research have shown the antibacterial efficacy of C. odorata leaf extract against a range of bacteria and fungi, as evidenced by the works of Aziz *et al*. (2020) and Elekofehinti *et al.* (2021). Notably, this extract has also exhibited action against keratinophilic fungi, as seen in the investigations conducted by Adeyemi *et al*. (2019) and Elekofehinti *et al.* (2021). The extract derived from *C. odorata* leaves has been shown to possess qualities that promote wound healing, as indicated by studies conducted by Aziz *et al*. (2020) and Elekofehinti *et al*. (2021). This suggests that it might potentially be beneficial in facilitating the restoration of injured skin resulting from keratinophilic fungal infections.

Nevertheless, there exists a dearth of extensive and methodical scholarly investigations pertaining to the efficacy of *C. odorata* leaf extract in combating keratinophilic fungal infections and promoting wound healing. Hence, the objective of this study is to assess the therapeutic efficacy of an extract derived from *C. odorata* leaves in treating keratinophilic fungal infections.

Materials and Methods

The isolation and characterization of keratiniphilic fungi from a soil sample.

The collection of soil samples was conducted in accordance with the methodology outlined in the research paper authored by Iheukwumere *et al.* (2021). The humus present on the surfaces of the soil was meticulously removed by using a sterile stainless spatula. The soil auger was used to excavate soil to a depth of 15 cm in the designated area, and a total of 10 soil samples were collected from each sampling unit and placed into a sterile basin. The samples underwent a comprehensive mixing process, during which extraneous components such as roots, stones, pebbles, and gravels were meticulously eliminated. The soil sample was then divided into two equal portions by a process known as quartering. The process of quartering included the division of the soil sample into four equal parts. Subsequently, the two quarters located opposite to each other were eliminated, while the remaining two quarters were combined by mixing. The aforementioned procedure was replicated for the other soil samples used in this investigation. The samples were meticulously labeled and thereafter stored in a sterilized cooler to ensure the preservation of their temperature and the stability of the isolate count. The specimens were conveyed to the laboratory for the purpose of analysis.

Isolation of Keratinophilic fungi

The isolation of keratinophilic fungi was conducted using the hair baiting approach, as outlined in the research conducted by Jangid and Begun (2018). In this study, Petri dishes of 90 mm x 90 mm were used. The soil samples were placed in the Petri dishes, with a volume of 10 g. To cover the surface of the soil, sterile defatted human or animal hair, which had not exceeded 20 years, was evenly distributed. A volume of 5 mL of sterile distilled water was then introduced to the soil, which was then incubated under controlled room temperature conditions (30 ±2°C) for a duration of 4 weeks, while being kept in darkness. The hair used in this research was sterilized by a multi-step process. Initially, the hair was subjected to many washes, commencing with

the application of a detergent (specifically, hair shampoo), followed by rinsing with water. Subsequently, the hair was treated with diethyl ether. Finally, the hair underwent autoclaving at a temperature of 1210C and a pressure of 15 PSI for a duration of 15 minutes. The colonies derived from the plates were then cultured on Sabouraud Dextrose Agar (SDA) supplemented with Chloramphenicol (50 mg/L) and cycloheximide (50 mg/L). The cultures were then kept at room temperature (30 ±2°C) for a duration of 7 days, as described by Aziz and Seema (2015).

The process of identifying fungal isolates

The identification of the fungal isolates was conducted using a combination of cultural and microscopic features seen in the pure cultures of the isolates.Iheukwumere *et al*. (2020). A thorough analysis was conducted on the colonies to assess their fungal attributes. The technique used for the observation of the pace of development, color, form, texture, consistency of the growth, and other distinctive characteristics of the colonies followed the approach outlined by Iheukwumere *et al*. (2021).

Microscopic characteristics:

The slide culture method mentioned in the study conducted by Iheukwumere *et al*. (2020) was used in this research. A piece of filter paper was cut and thereafter positioned at the base of a Petri dish. Two slides, each measuring 75 mm x 26 mm x 1 mm, were placed in a crossing configuration on top of a Whatman No 1 filter paper measuring 110 mm. The filter paper was then wet. The setup underwent sterilization using autoclaving at a temperature of 121 degrees Celsius for a duration of 15 minutes. A square agar block of about one centimeter in length on each side was excised from a pre-prepared medium known as Potato Dextrose Agar (PDA). The block was thereafter positioned at the point of intersection between two glass slides. The test organisms were injected on the four sides of the agar block. Subsequently, a sterile cover slip measuring 22 mm × 22 mm was placed over it, and the sample was incubated at room temperature (30 ±2°C) for a period of 7-10 days. Following the completion of growth, the cover slip was carefully detached and then inverted onto a slide that contained a little amount of lactophenol cotton blue (LCB) solution. The agar block was subsequently extracted and disposed off. Furthermore, a single droplet of LCB was applied onto the surface of the adhering colony situated on the slide, followed by the placement of a sterile cover slip. To mitigate the potential evaporation of the stain, the periphery of the cover slip was sealed using nail paint. The slides were subjected to microscopic examination with objective lenses with magnifications of x10 and x40.

Pathological Features of the Keratiniphilic Fungi

The present study focuses on the pathological characteristics shown by the keratiniphilic fungi. The test isolate used in this investigation is of particular interest. The test isolate was generated by combining 0.05 mL of a 1% $BaCl_2.2H_2O$ solution with 9.95 mL of a 1% concentrated H_2SO_4 solution, resulting in a 0.5 McFarland matching standard. The test isolate was produced using a solution of normal saline containing 0.85% sodium chloride (NaCl). The culture plates were immersed in normal saline solution and then subjected to extensive maceration and filtration using Whatman No 1 filter paper. The filtrate was then diluted to achieve a turbidity level equivalent to the manufactured 0.5 McFarland standard, which contained 1.5×10^8 cells per milliliter.

Experimental animal:The subject of investigation in this study is an animal model used for experimental purposes. The laboratory animals used in this investigation were albino Wistar rats procured from the animal house at the University of Nigeria, Nsukka (UNN). The rats were sent to the animal facility located at the Department of Biochemistry, inside the Faculty of Bioscience at Nnamdi Azikiwe University (NAU) in Awka. The rats underwent a random selection process to assess their appropriateness for inclusion in the research. Individuals who were deemed unsuitable for the test were eliminated from the research.

The present investigation focuses on an examination of animal behavior and physiology.

Animal study: The experiment was conducted using the adapted technique outlined in the research paper authored by Iheukwumere *et al*. (2021). This research used a cohort of 32 albino Wistar rats. Each rat's body was disinfected using a solution of 70% ethanol. The rats were divided into four groups, with each group consisting of eight rats. The rats in groups one, two, and three were subjected to topical infection with 0.5 mL of organisms D, E, and F, respectively. The fourth group was used as a control group without any infection. The pathogenic characteristics of the test isolates were observed and recorded throughout a duration of 56 days.

The process of preparing plant materials

The researchers procured the recently harvested leaves of *Chromolaena odorata* from a cultivated area located in Amawbia, Awka, Anambra State, Nigeria. The provided sample underwent proper authentication procedures. The samples were subjected to a drying process in a shaded environment at a consistent room temperature of 30±2℃ for a duration of 21 days. The dried leaves were pulverized into a fine powder using a sterilized electric grinder (LXB 242/LE Max). A quantity of twenty grams (20 g) of ground leaves was subjected to maceration in ethanol (99%) for a duration of 72 hours. The combination underwent filtration using a Whatman No 1 filter paper with a diameter of 110 mm. The extract was subjected to concentration by evaporation to complete dryness at room temperature under a continuous air current, as described by Iheukwumere *et al.* (2018).

Therapeutic Potential of the Prepared Cream against Superficial Infection caused by Keratiniphilic Fungi

The present study focuses on the formulation of a cream using the extract derived from *Chromolaena odorata* leaves.The organic cream was created by using a modified version of the methodology outlined in the research article authored by Iheukwumere *et al.* (2021). The primary alteration in the current investigation mostly pertained to the active component and the amount used. The shea butter, measuring 500 mL, and almond oil, measuring 50 mL, were combined inside a stainless steel saucepan with a capacity of 2 liters. The mixture was vigorously agitated and subjected to heat from a cooking gas source until all the constituents were fully liquefied. Subsequently, the incorporation of the extract (200 mL) into the mixture ensued, and thorough agitation and blending were performed. Subsequently, the concoction underwent fortification with vitamins A, C, D, and E, as well as the addition of a colorant and aroma. The cream was then distributed into a container, with a volume of 20 mL, and left to harden. The prepared cream's therapeutic potential against superficial infections caused by keratinophilic fungi was evaluated.

This research used a sample size of 24 albino Wistar rats exhibiting dermatological lesions resulting from the test isolates in the pathological investigation. The rats were divided into three groups, each consisting of eight rats, depending on the three keratinophilic fungi that were investigated in this research. Each group of rats received a topical application of 1.0 g of the cream three times each day, namely in the morning, afternoon, and night. The dermatological lesions present on the rats under study were observed over a span of 56 days to assess their healing capability.

Statistical Analysis

The data obtained in this study were presented in tables. Chi square(x^2) was used to determine the significance of the sample sources 95 % confidence level. Pairwise comparison was carried out using student "t" test (Iheukwumere *et al*. 2021).

Results and Discussion

Table 1 displays the macroscopic and microscopic characteristics of the keratinophilic fungus isolates. Isolate D had a whitish hue and has a powdery texture, characterized by a substantial presence of macroconidia. The presence of microconidia was observed, although in limited quantities, exhibiting a thick and club-shaped morphology. Isolates E and F had a white to cream coloration and displayed a development rate ranging from slow to moderate. These isolates were characterized by the presence of many microconidia that were thin and possessed a smooth surface. The microconidia seen in isolate E had a thin or club-shaped morphology, whereas those in isolate F displayed a spherical morphology. The texture of the colony of isolate E was found to be smooth, whereas the texture of isolate F was observed to be powdery. Isolate F exhibited the presence of clavate, thin, and smooth macroconidia, but isolate E lacked this characteristic.

Table 1. Macroscopic and microscopic characteristics of the keratinophilic fungal isolates

Parameter	D	E	F
Colour of Colony	Beige	White/Cream	White
Texture	Powdery	Downy	Powdery
Reverse Colour	Yellow	Yellow-brown	Reddish-brown
Growth Rate	Rapid	Slow/Moderate	Moderate
Macroconidia	Present/Abundant	Absent	Present but scanty
Shape of macroconidia	Fusiform with rounded ends	_	Clavate
Walls of macroconidia	Thin and rough	_	Thin and smooth
Microconidia	Present/scanty	Present/Abundant	Present/Abundant
Shape of microconidia	Club-shaped	Slender/Club-shaped	Spherical
Walls of microcidia	Thick	Thin	Smooth
Hyphae	Septate	Septate	Septate
Fungus	Microsporum gypseum	Trichophyton rubrum	Trichophyton mentagrophytes

Pathological Features of the Isolated Keratinophilic Fungi

The investigation revealed that the keratinophilic fungi that were isolated had diverse pathogenic characteristics, as shown in Table 2. All of the rats that were infected had symptoms of alopecia, erythema, and discoloration. The rats infected with TS exhibited a higher prevalence of pathological characteristics, including papule, macle, bulla, and ulceration. In contrast, the expression of these pathological features varied among rats infected with MS and TRS. The study also demonstrated that there was a statistically significant ($P < 0.05$) presence of pathological features in the rats infected with kertinophilic fungi compared to the control group. However, among the infected rats, the occurrence of these pathological features was not statistically significant ($P > 0.05$).

Table 2: Pathological features of the isolated keratinophilic fungi

Parameter		N= 8		
	C	MS	TS	TRS
Alopecia	0	8	8	8
Erythema	0	7	8	8
Discolouration	0	8	8	8
Papule	0	3	4	2

Bulla	0	2	4	2
Ulceration	0	1	3	2
Macule	0	3	4	3

C = Control (Normal); MS = *Microscoporum spp*; TS= *Trichophyton spp;* TRS = *Trichophyton spp*

Characteristics of the Prepared Antifungal Cream

The antifungal cream that was made had a yellow hue and possessed the typical attributes of a cream. The cream exhibited a smooth and translucent appearance, with a viscous texture that aligns with the favorable pH level for skin, as shown in Table 3. Table 4 displays the absence of microbial counts for several bacterial species, including total heterotrophic aerobic bacteria (THABC), total coliform (TCC), faecal coliform (TFCC), *Staphylococcus aureus* (TSC), *Salmonella-Shigella* (TSSC), as well as fungal species such as molds (TMC) and yeasts (TYC). Additionally, anaerobic bacteria (TAC) were also not detected.

Table 3: Physical characteristics of the prepared antifungal cream

Parameter	Inference
Colour	Yellow
Surface	Smooth
Texture	Thick
Optical Nature	Transparent
Viscosity	46.70 mPa.S
Specific gravity	0.904
Refractive Index	1.460
pH	7.30

Table 4: Microbial quality of the prepared antifungal cream

Parameter	Value (CFU/g)
TCC	0
TFCC	0
TSC	0
TSSC	0
THABC	0
TMC	0

TYC	0
TAC	0

TCC= Total coliform count; FFCC= Total faecal coliform count; TSC= Total *Salmonella –Shigella* count; THABC= Total Heterotrophic Aerobic Bacterial Count; TMC= Total MoldCount; TYC= Total Yeast Count; TAC= Total Anaerobic Bacterial Count

Therapeutic Potential of the Prepared Cream against the Keratinophilic Fungal Infection

The antikeratinophilic fungal cream that was developed shown significant therapeutic efficacy against the infections induced by MGS, TS, and TRS, as seen in Tables 5, 6, 7, and 8. The research findings demonstrated the therapeutic efficacy of the cream in treating keratinophilic fungal infections. The observed healing capacity reached statistical significance ($P < 0.05$) after a duration of 28 days, with the most notable effects seen after 56 days. Additionally, it was noted that the coloration and erythema exhibited quick resolution in the rats subjected to the trial, whereas baldness was also detected.

Table 5: Therapeutic potential of the prepared cream against infections caused by MS

		Days								
Parameter	MS	7	14	21	28	35	42	49	56	C
Alopecia	8	8	6	6	5	5	3	3	2	0
Erythema	8	6	3	1	1	1	0	0	0	0
Discolouration	8	4	2	1	0	0	0	0	0	0
Papule	3	2	1	0	0	0	0	0	0	0
Bulla	2	2	2	2	2	2	1	1	0	0
Ulceration	1	1	1	1	1	1	1	1	0	0
Macule	3	3	2	2	2	1	0	0	0	0

Table 6: Therapeutic potential of the prepared cream against infection caused by TS

		Days								
Parameter	TRUS	7	14	21	28	35	42	49	56	C
Alopecia	8	8	8	7	6	6	4	4	3	0
Erythema	8	7	6	4	3	1	1	0	0	0

Discolouration	8	3	1	1	0	0	0	0	0	0
Papule	4	4	2	2	1	1	0	0	0	0
Bulla	4	4	4	4	3	3	2	1	1	0
Ulceration	3	3	3	3	3	3	3	1	1	0
Macule	4	4	2	2	1	0	0	0	0	0

Table 7: Therapeutic potential of the prepared cream against infection caused by TMS

		Days								
Parameter	TRS	7	14	21	28	35	42	49	56	C
Alopecia	8	8	8	8	7	4	3	1	0	0
Erythema	8	7	4	1	1	0	0	0	0	0
Discolouration	8	5	3	1	0	0	0	0	0	0
Papule	2	2	2	2	2	0	0	0	0	0
Bulla	2	2	2	2	2	2	2	1	0	0
Ulceration	2	2	2	2	2	2	2	2	0	0
Macule	3	3	3	2	2	1	1	0	0	0

Macule	0	3	0	4	0	3	0

Table 8: Summary of the therapeutic potential of the cream against keratinophilic fungal infection after 56 days

Parameter	C	MS	TMG	TS	TTR	TRS	TTM
Alopecia	0	8	2	8	3	8	0
Erythema	0	8	0	8	0	8	0
Discolouration	0	8	0	8	0	8	0
Papule	0	3	0	4	0	2	0
Bulla	0	2	0	4	1	2	0
Ulceration	0	1	0	3	1	2	0

Discussion

The characteristics exhibited by the isolated strains of *Microscoporum spp*, *Trichophyton spp*, and *Trichophyton spp* were consistent with the findings reported by several researchers (Vyas and Sharma, 2019; Iheukwumere *et al.*, 2020; Keta *et al.*, 2020; Kumawat *et al.*, 2020). The identification of *Microsporum spp*, *Trichophyton spp*, and *Trichophyton spp* in this study aligns with the results reported by Sharma and Choudhary (2015), Jangid and Begun (2018), Vyas and Sharma (2019), Iheukwumere *et al.* (2020), and Kumawat *et al.* (2020). However, it contradicts the findings of Nosratabadi *et al*. (2017). According to the findings of Vyas and Sharma (2019), the fungal strains M*icroscoporum* and *Trichophyton* were often identified as keratinophilic fungi and dermatophytes. These fungal species have ecological significance and contribute significantly

to bioremediation processes in natural environments. Several studies (Jangid and Begun, 2018; Iheukwumere *et al*., 2020; Kumewat *et al*., 2020) conducted the isolation and characterization of *M. gypseum, T. rubrum*, and *T. mentagrophytes* from soil samples. According to Iheukwumere *et a*l. (2020), the presence of keratinophilic fungus in the soil samples may be linked to the high abundance of keratinous materials, as well as the prevailing conditions of temperature, humidity, and pH in the soil samples. Nwokeoma *et al.* (2017) and Keta *et al.* (2020) documented the presence of *Microscoporum* species and *Trichophyton* species, as well as non-dermatophytes, as keratinophilic fungi that were isolated from various soil samples.

The observed pathological lesions related to the keratinophilic fungus under investigation align with the discoveries made by other researchers (Mark *et al.*, 2015; Claire *et al*., 2016; Iheukwumere *et a*l., 2020). The occurrence of pathological lesions may be linked to the fungi's capacity to break down the keratinized portion of the skin, facilitated by the release of an extracellular proteolytic enzyme called keratinase. This enzyme's synthesis seems to be driven by the presence of keratin in the tissue or substrate. The review released by Filipello (2020) also presented a similar deduction. The pathological lesion severity attributed mostly to *Trichophyton* specie(TRS) in this investigation aligns with the conclusions drawn by Jangid and Begun (2018) as well as Iheukwumere *et al.* (2020). However, it contradicts the results reported by Mark *et al*. (2015) and Theresa *et al*. (2019). According to Filipello's (2020) published review, the extent of keratinization may be responsible for the severity of the pathogenic lesions.

The properties of the created cream, including its texture, surface features, optical properties, viscosity, specific gravity, refractive index, and pH, align with the fundamental and standardized parameters expected of an ideal cream. In previous studies conducted by Inoue *et al*. (2014) and Chen *et al.* (2016), same conclusions were reached. The absence of coliform, faecal coliform, *Salmonella-Shigella*, heterotrophic aerobic bacteria, anaerobic bacteria, yeast, and mold counts in the cream indicates that it is sterile and meets the basic microbiological quality requirements set by regulatory authorities.

The results of Iheukwumere *et al.* (2021) provided evidence for the considerable therapeutic potential of the cream. The cream's therapeutic efficacy may be ascribed to the phytochemical and mineral constituents included in the extract derived from *Chromolaena odorata* leaves, which serves as the cream's active ingredient. Iheukwumere *et al*. (2021) also presented a comparable study. In a study, Chen *et al.* (2016) documented that the extract of *C. odorata* stimulates the production of heme oxygenase-1 and thromboxane synthase. Additionally, they observed a reduction in the levels of the vasoconstrictor and anti-platelet aggregator matrix metallopeptidease 9 (MM9). These findings suggest that the extract promotes homeostatic processes and facilitates wound healing.

Conclusion

The present study has demonstrated that the keratinophilic fungi, specifically *Microsporum species* (MS), *Trichophyton specie*(TS), and Trichophyton specie(TRS), induced diverse pathological lesions on the skin of albino Wistar rats used in the experiment. However, these lesions were effectively treated using a cream formulation containing an extract derived from the leaves of *Chromolaena odorata*. Hence, our research proposes that the formulated cream might serve as a viable treatment option for treating topical infections induced by keratinophilic fungus.

References

Adeyemi O.S., Olayide I.I., Olasehinde G.I., & Arojojoye O.A. (2019). Antifungal activity of Chromolaena odorata leaf extract against selected dermatophytes. *Journal de Mycologie Médicale/Journal of Medical Mycology* , 29(3), 223-227.

Aziz M.A., Rahman M.M., Islam M.T., Uddin M.E., Shilpi J.A., & Uddin S.J. (2020). Chromolaena odorata (L.) R.M.King & H.Rob.: A review on traditional uses, phytochemistry and pharmacology. *Journal of Ethnopharmacology* , 247(112270).

Chen, M.X., Alexnder, K.S. and Baki, G. (2016). Formulation and evaluation of antibacterial creams and gel containing metal ions for topical application. Journal of Pharmaceuticals 4016: 1 – 10.

Elekofehinti O.O., Adanlawo I.G., & Komolafe K. (2021). Antifungal activity and wound healing potential of Chromolaena odorata leaf extract in guinea pigs infected with Microsporum gypseum. *Journal de Mycologie*

Médicale/Journal of Medical Mycology , 31(1), e101020.

Filipello, M.V. (2020). Keratinophilic fungi: Their role in nature and degradation of keratinic substrates. Retrieved from www.researchgate.net/publication, 28th June 2023, pp. 1 – 8.

Iheukwumere, I.H., Opara, G.R., Iheukwumere, C.M., Okafor, C.F. and Nwakoby, N.E. (2021). Prophylactic potential of essential cream produced from Chromolaena odorata leaf extract against Cladosphialophora bantiana strain D12E. IPS Journal of Applied Microbiology and Biotechnology 1 (1): 1 – 11.

Gupta A.K., Daigle D., & Foley K.A. (2017). Antifungal agents: mechanisms of action. Expert Review of Anti-infective Therapy , 15(6), 557-566.

Iheukwumere, I.H., Dimejesi, S.A., Iheukwumere, C.M., Chude, C.O., Nwaolisa, C.N., Ukoha, C.C., Nwakoby, N.E., Egbuna, C. and Egbe, P.A. (2020). Diversity and molecular characterization of keratinophilic fungi from soil smples. International Journal of Research Publication 50 (1): 1047 – 1062

Iheukwumere, C.M., Iheukwumere, I.H., Chude, C.O., Egbe, P. and Egbuna, C. (2020). Sequential pathological scales of keratinophilic fungi isolated from geophilic soil samples. European Journal of Biomedical and Pharmaceutical Science 7(5): 48 – 53

Inoue, Y., Suzuki, K., Maeda, R., Shimura, A., Murata, I. and Kanamoto, I. (2014). Evaluation of formulation properties and skin penetration in the same additive containing formulation. Results Pharmaceutical Sciences 4: 42 – 49

Jangid, R. and Begun, T. 92018). Isolation and identification of keratinophilic fungi from soil samples of different animal habitat of Ajmer district, India. International Journal of Pure and Applied Bioscience 6 (2): 646 – 652

Keta, J.N., Shinkafi, S.A., Abubakar, M., Aminu, M., Keta, M.N., Gudu, G.J. and Patrick, R.J. (2020). Prevalence of keratinophilic fungi from water logged condition of paddy soil in Bgudo metropolis, Kebbi State, Nigeria. Journal of Innovative Research in Life Sciences 2(1): 1 – 6

Kumawat, T.K., Sharma, A., Sharma, V., Chandra, S. and Bhadauria, S. (2020). A study on the prevalence of keratinophilic fungal biota of semi-arid region of Rajasthan, India. Journal of King Saud University – Science 32 (1): 1014 – 1020

Mark, T.W., Nicole, B., James, G. and Stephen, W.B. (2015). Biology and Diseases of mice, Third Edition. Elsevier Incorporation, California, pp.143 – 149 .

Mishra A.K., Singh S.K., Pandey A.K., & Tripathi V.D. (2018). Zoonotic dermatophytosis (ringworm): an overview. Journal of Infection and Public Health , 11(6), 779-787.

Nosratabadi, M. Kordbacheh, P., Kuchei, R., Safara, M., Rezaie, S. and Afshari, M.A. (2017). Isolation of keratinophilic fungi from the soil of Greater Tunb, Abu – Musa, and Sirri islands in the Persian Gulf, Iran. Current Medical Mycology 3 (2): 13 – 19

Nwokeoma, C.I., Tasie, F.O., Akinyele, H.A., Samuel-Osamoka, F.C. and Osanyinlusi, S.A. Keratinophilic fungi associated soils from selected villages in Ugwunye and Ojebogene towns, Enugu State, Nigeria and their disease implications. The Bioscientist 5 (1): 20 – 31.

Sahni N., Sharma S., & Prakash A. (2019). Keratinophilic fungi: a review. *Journal of Fungi* , 5(4), 100.

Sharma, R. and Choudhary, N. (2015). Isolation of keratinophilic fungi from soil samples of agricultural fields of Saharanpur, India. International Journal of Current Microbiology and Applied Sciences 4 (7): 229 – 237 .

Theresa, B., Agnes, F., Candy, G., Silke, U., Tilo, H., Pietro, N., Christoph, G.B. and Wieland, S. (2019). Trichophyton benhamiae and T. mentagrophytes target guinea pigs in a mixed small animal stock. Medical Mycology Case Report 23: 37 – 42.

Vyas, S. and Sharma, N. (2019). Isolation and identification of keratinophilic fungi from region park of Indore. Journal of Emerging Technologies and Innovative Research 6 (6): 826 – 828.

IJORP