

EFFECT OF TEA LEAVES EXTRACT IN DANDRUFF CAUSING MICROBES

(Project – Report)

Submitted in partial fulfillment of the requirements for the award of

Bachelor of Technology degree in biotechnology

By

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(DEEMED TO BE UNIVERSITY)

Accredited with Grade “A” by NAAC

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of RUBIKA.L and S.Susmitha who carried out the project entitled “ **EFFECT OF TEA LEAVES EXTRACT IN DANDRUFF CAUSING MICROBES** “ under our supervision from December 2020 March 2021

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DECLARATION

I **RUBIKA.L** hereby declare that the Project Report entitled

“EFFECT OF TEA LEAVES EXTRACT IN DANDRUFF CAUSING MICROBES” done by me under the guidance of **Dr. Sudha** at **Sathyabama institute of Science and Technology, Chennai** is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

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ABSTRACT

Dandruff is a common disorder affecting the scalp condition. Dandruff can't be absolutely eradicated however can solely be managed and effectively controlled. Currently on hard products in the market have terrible effectively or recurrence of symptoms. Plant extracts possess a range of energetic compounds that might also assist to overcome emergence of resistance in causative organism. In the existing study, an attempt was once made to understand the activity of tea plant extracts against the dandruff causing organism *Malassezia globosa*, *M.restricta*. Tea plants were collected and their crude as well as powered extracts were used for the activity studies. These results were compared with the antifungal activity of three branded shampoos. On comparison, it is inferred that plant extracts confirmed promising activity against *Malassezia globosa*, *M.restricta* when compared with the marketed antidandruff shampoos.

Key words: Antidandruff shampoo, Antifungal activity, Dandruff; *Malassezia globosa*, *M.restricta*.

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LIST OF ABBREVIATIONS AND SYMBOLS:

gms	-	Grams
ml	-	Millilitre
min	-	Minute
mg	-	Milligrams
mg/ml	-	Milligram per millilitre
ug/ml	-	Microgram per millilitre
°C	-	Degree celcius
cm	-	Centimetre
%	-	Percentage
SDA	-	Sabouraud Dextrose Agar
H ₂ O ₂	-	Hydrogen peroxide
MIC	-	Minimum inhibitory concentration
MDR	-	Multiple drug resistance

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CHAPTER1: INTRODUCTION

1.1 What is dandruff microorganism?

A crew from Japan and China found dandruff, which afflicts around 1/2 the world's population, is extra strongly linked to populations of two bacterial agencies – *Propionibacterium* and *Staphylococcus* – than the *Malassezia* fungus, which additionally fortunately colonises the human scalp and is broadly believed to be the main cause of the condition. Most dandruff studies have focused on *Malassezia*. There are 14 known species of *Malassezia*, with two – *M. restricta* and *M. globosa* – linked to skin diseases.

But they're not the only microbes living in your hair. Bacteria like it there too. It's warm and full of food. Fungi and bacteria chow down saturated fatty acids discoverd in sebum, the oily substance secreted from the scalp.

So Menghui Zheng from Shanghai Jiao Tong University in China and colleagues swabbed the scalps of 59 Chinese guys and women, aged 18 to 60, 48 hours after washing with a non-anti-dandruff shampoo to confirm sebum levels and bacterial and fungal populations.

1.2 *Malassezia globosa*, *M. restricta*

They are 15 species of *Malassezia* genus unicellular basidiomycete yeast species, namely *Malassezia globosa*, *Malassezia restricta*, *Malassezia furfur*, *Malassezia sympodialis*, *Malassezia slooffiae*, *Malassezia dermatis*, *Malassezia pachydermatis*, *Malassezia equina*, *Malassezia caprae*, *Malassezia cuniculi*, *Malassezia nana*, *Malassezia obtusa*, *Malassezia japonica*, *Malassezia yamatoensis*, and *Malassezia arunalukei*. The first six are the most commonplace fungi constituting the fungal microbiota of human skin. Rarely, some species of *Malassezia* can motive IFIs by capacity of producing hyphae, which regularly have an effect on the coronary coronary coronary heart and lungs in hospitalized infants receiving parenteral nutrition and in younger victims due to the unsuitable use of catheters [. Conversely, seborrheic dermatitis, atopic dermatitis, folliculitis, pityriasis versicolor, and dandruff can normally occur, with *Malassezia* spp. being the essential etiologic agent. In particular, dandruff is an disagreeable unpleasant condition, affecting greater than 50% of the world's population . The triggering elements are multiplied sebum production, individual susceptibility (inheritance), and irritation determined through the use of pathogenic microorganisms, such as *Malassezia* spp. Initially, the etiology of pathogenic dandruff used to be attributed to *M. furfur*, then again because of this it emerged that *M. globosa* and *M. restricta* present in the scalp are in most situations responsible. Because these fungi decide upon a lipid-rich surroundings as the major carbon supply for growth, sebum is first-rate for colonization by *Malassezia* spp. Hypersecreted fungal lipases liberate the fatty acid precursors from the triglycerides modern-day in the cutaneous sebum. The latter are hence absorbed, marketing unbridled myristic acid synthesis quintessential for the fungus . In particular, oleic acid was once once tested to be the indispensable product fashioned with the hydrolysis of triglycerides by *M. globosa* lipases. Oleic acid penetrates the dermis corneum layer and motives an inflammatory response

in the most sensitive individuals, ensuing in accelerated spreading and dispersion of lifeless pores and skin cells .

Electron microscopy research have tested that the cells of *Malassezia* spp. have a structurally thick and multilaminar cell wall with a attribute invagination. The immoderate lipid percentage of the fungal wall (15%–20% w/w), accelerated than *S. cerevisiae* and *C. albicans*, makes it even more hydrophobic and in charge for resistance to drugs and the host immune system as accurate as being the infection trigger . Recent search for have verified that infection can be additionally brought about by way of the manufacturing of adhesion molecules to human cutaneous keratinocytes, chemokines, and fungal inflammatory cytokines . Moreover, the presence of the allergenic protein Mala s 1 on the fungal wall has been implicated in pores and pores and skin disorders .

The on hand antidandruff recovery approaches consist of incorporating lively elements in shampoos to minimize the fungal increase by way of inhibition of sterol /biosynthesis (ketoconazole or one of a sort azoles), or by using interrupting membrane things to do (zinc pyrithione) . Other lots much less fantastic redress are based definitely on selenium disulfide, coal tar, or sulfur .

Malassezia belongs to the division of fungi Basidiomycota in the kind Exobasidiomycetes (those observed out of doors the host body) and in the order Malasseziales. They have been first off recognized by using way of a French scientist Louis

Charles Malassez in the nineteenth century. Later Raymond Saboraud recognized the dandruff-causing organism in 1904 and named it *Pityrosporum malassez*, honoring Malassez but at the species level. When it was once once determined out that the

organisms had been the same, the time length “*Malassezia*” used to be judged to posses precedence (

Dandruff is a circumstance characterised thru potential of flaking of pores and skin (most commonly scalp skin) resulting from fast turnover and launch of pores and skin cells.

Dandruff is reliant on three (3) factors that desire its survival and copy of the yeasts, they are:

- *Sebum production
- * Microbial metabolism
- * Susceptibility of individual.

Recently, identification of *Malassezia* on skin has been aided through the software of molecular or DNA primarily based techniques. These investigations exhibit that the *Malassezia* species inflicting most pores and skin diseases in human beings

the most common motive of dandruff and Seborrhoeic dermatitis is *M. globosa*. The skin rash of *Tinea versicolor* is also due to this fungus. As the fungus requires fats to grow, it is most many times observed in areas with many sebaceous glands: on the scalp, face and higher section of the body. But when the fungus grows too hastily, the natural renewal of cells is disturbed and dandruff appears with itching (a similar procedure may also show up with different fungi or bacteria). The yeast produces components that irritate and inflame the skin. Patients with Seborrhoeic dermatitis show up to have a decreased resistance to the yeast. However, the colonization charge at affected pores and skin may also be lower than that of unaffected skin. Only saturated fatty acids have been shown to assist *Malassezia* growth. It has additionally been proven that while range density of *M. globosa* do no longer without delay correlate to dandruff presence or severity, elimination correlates at once with amelioration of flaking.

Furthermore, in dandruff inclined individuals, pure oleic acid, and unsaturated fatty acid and *Malassezia* metabolites set off flaking in the absence of *Malassezia* by direct effects on the host's pores and skin barrier. Genetic, environmental, hormonal and immune gadget factors have been shown to be worried in the manifestation of Seborrhoeic dermatitis. Seborrhoeic dermatitis may additionally be aggravated through illness, physiological stress, fatigue or sleep deprivation. Dandruff is the shedding of useless pores and skin cells from scalp). Dandruff is a common scalp sickness affecting almost half of of the population at the post-pubertal age and of any gender and ethnicity. It often motives itching. It has been well mounted that keratinocytes play a key function in the expression and technology of

immunological reactions throughout dandruff formation. The severity of dandruff might also fluctuate with season as it often worsens in winter .

Dandruff is characterised by patches of loosely adherent flakes, typically accompanied by itching. Dandruff has the clinical function of small white or gray flakes that accumulate diffusely on the scalp in localized patches. It does not exhibit apparent inflammation and is confined to the scalp. For most individuals, as the epidermal layer constantly replaces itself, cells are pushed outward where they finally die and flake off. These flakes of pores and skin are too small to be visible. However, certain prerequisites cause mobile turnover to be unusually rapid, especially in the scalp. For humans with dandruff, skin cells might also mature and be shed in 2–7 days, as opposed to around a month in human beings barring dandruff. The end result is that dead skin cells are shed in large, oily clumps, which appear as white or grayish patches on the scalp, pores and skin and clothes . Dandruff has been proven to be the result of three required factors are :

1. Skin oil generally referred to as sebum or sebaceous secretions
2. The metabolic by-products of skin micro-organisms (most specifically *Malassezia* yeasts).
3. Individual susceptibility Sebaceous gland pastime suggests strong association

with most disorders that purpose scalp flaking.

4. Early sebum production in the neonate can also occur as cradle cap. Once maternal hormone manipulate subsides, sebum manufacturing does not commence again until puberty, at which time sex hormone manage impacts sebum production. As sebum production increases, *Malassezia* proliferates in response to new food sources. Proliferation of *Malassezia* will increase lipid metabolism, which reasons scalp itching and flaking. *Malassezia* species are lipid-dependent microorganisms that adapt to the narrow area of interest furnished with the aid of sebum-rich skin. *Malassezia globosa* and a 2nd species *M. restricta* predominate on dandruff scalp.

5. *M. globosa* possibly initiates dandruff formation due to its lipase activity. *M. globosa* lacks the ability to synthesize fatty acids: it is exceedingly adaptive however niche structured and is oftentimes found on the scalp, back, face and chest the place the best possible ranges of sebum are produced. *M. globosa* excretes extra than 50 specific enzymes to help metabolize hair and scalp.

6. *Malassezia* metabolism effects in improved oleic acid ranges that lead to the signs and symptoms of dandruff and different varieties of dermatitis in some individuals. It had been beforehand referred to that the fungus *Malassezia furfur* (previously recognised as *Pityrosporum ovale*) as the reason of dandruff. While this species does manifest naturally on the pores and skin surface of each healthy humans and these with dandruff, it was found in 2007 that the responsible agent is a scalp particular fungus .

7. *Malassezia globosa* that metabolizes triglycerides existing in sebum with the aid of the expression of lipase, ensuing in a lipid byproduct, the oleic acid (OA). During dandruff, the degrees of *Malassezia* enlarge through 1.5 to 2 instances its normal level. Penetrating the top layer of the epidermis and the stratum corneum, through Oleic acid (OA), consequences in an inflammatory response in susceptible men and women which disturbs homeostasis and results in erratic cleavage of stratum corneum cells . studied a protocol of the biochemical, morphological and molecular characteristics of *M. globosa* and its consent varieties have been approved via the research and ethic committee of Imandade Santa Case de Misericordia de Sau Paulo. He classified the fungal physique into Light (23%); Moderate (56.86%); Decimated (19.60%). The objective of this mission used to be to isolate and identify the fungus *M. globosa*, the dandruff-causing the usage of the gorgeous methods employed, with a view to discovering the excellent therapy to the incessant scalp and hair flaking and loss as complained through the girl college students of the university.

CHAPTER 2: REVIEW OF LITERATURE

Mamatha Pingili, Sridhar Vanga, Ramakrishna Raparla november 2016

Plant extracts showed good activity against dandruff causing organism *Malassezia furfur*. From the results, we conclude that plant extracts have

antifungal activity and could be safely used for treating dandruff. Further studies can be made on the active molecules of plant extracts responsible for antidandruff activity. Toxicity evaluation of these extracts can also be carried out. Synthetic

drugs are unable to prevent recurrence [2]. Recurrence of dandruff up on usage of these plant extracts can also be explored.

Prabha Manju M, Gokulshankar S ,Navin Kumar Sharma ,Babu K ,Chiranjeevi A October 2012, compounds based on the knowledge that plants have their own defense systems against fungal pathogens. Our current study reports the use of herbal actives against dandruff. In conclusion, the above plants extract and polyherbal oil shows promising activity against *M. globosa*. Detailed studies on combination of formulations and in vivo studies are however required to confirm the functional anti-dandruff activity of the product.

Sreelatha Gopalakrishnan Lalitha, Lakshmeesha Thimappa Ramachandrappa, Soumya Krishnamurthy, Jayashree Basavaraju, Sharmila Thirumale July 2016, To conclude, it is imperative to emphasize the significance of more comprehensive studies that will detect new lead molecules underlying the action of crude extracts that allow for new discoveries. Essentially, the present study, which establishes the lack of significant antimalassezial activity, removes the need for any repetitive study with these extracts in the future.

Sonia Angeline M, Sushmitha.G. Helavar, Megha Sirohia dandruff is a chronic scalp condition characterized by scaling, itching and redness of the scalp. It occurs when dead skin cells are shed in large oily clumps, which appear as white or grayish patches on the scalp, skin and clothes called dandruff. *Malassezia* is a monophylic and unipolar lipophilic yeast. It is a part of natural body flora and associated with a variety of conditions including dandruff, atopic dermatitis, pityriasis versicolor, seborrheic dermatitis and folliculitis [18]. The dandruff causing fungi was cultured in different fungal media such as PDA, SDA, and DA, the colonies in Dixon agar were observed as creamish, round, mucoid and smooth regular concave surface. In Potato Dextrose agar, the colonies were observed as pale white, round, mucoid and smooth regular concave surface. In Sabouraud Dextrose agar the colonies were observed as white, yellow and orange in colour, round shape, mucoid and smooth regular concave surface. In our study we

analyzed the growth of fungus in different fungal media and also by modulating the normal pH range followed by incubating the fungi in two different temperature conditions as discussed earlier. These studies were done to check the growth of fungus at varied pH and temperatures. It was found that by incubating at different environmental conditions, dandruff causing fungi were able to grow faster in incubator within 24 hours where as in room temperature the growth was observed after 2 days. It was observed that at pH below 4.5 the growth of dandruff causing fungus was less and at pH above 7.0 the growth of dandruff causing fungus was more. In Dixon agar by altering the normal pH range the colony morphology did not change while in PDA and SDA the colony morphology were different. The aim is to study the effectiveness of fruit peel extracts because they contain broad spectrum of phenolic compounds and vitamins which have the ability to overcome the resistance in causative organism. Citrus fruits are rich in vitamin C which maintains the pH level of scalp and prevents itching and shedding of flakes. Many fruit peel extract have been used and tested to check their therapeutic activity. From the study the antifungal activity of the fruit peel extract was judged by performing zone of inhibition method. The samples were collected from the scalp of different individual to conduct the study. The fruit extract was obtained by soaking the powdered fruit peel with methanol. Orange, sweet lime, lemon, grape were evaluated for the physicochemical characteristics. The results were significant at different interval. Among all the citrus fruit lemon showed high content of citric acid but grapes and orange were rich in total soluble solids [19]. Among all the fruit peel extracts used, lemon peel extract was able to inhibit in all the concentrations (20 μ l -100 μ l), followed by orange which was able to inhibit at 40 μ l, 80 μ l and 100 μ l concentration in SDA only. NMR analysis was carried out which revealed that Vitamin C is the compound present in lemon fruit peel extract which was found to have anti dandruff activity. Therefore, natural products have relevant advantages over synthetic compounds they are easily available, comparatively cheaper, and can reduce side effects and irritation potential of chemicals to a large extent. Plants are rich in wide variety of secondary metabolites such as tannins, alkaloids and flavonoids found to have antimicrobial properties [20]. Our study helps in choosing the advisable fruit peel extract with anti-dandruff activity which is easily available and helps in assessing the grade of new products containing lemon as an active compound

Meena Deviha S., PavithraM.K.S, All the plant extracts showed a significant antidandruff activity and also been used traditionally to induce hair growth. The results obtained from the work are encouraging and would probably help to make a polyherbal mixture which find its application in inducing hair growth activity.

CHAPTER 3 : AIM AND SCOPE

3.1 AIM :

To elucidate and evaluate the tea leaves(**Camellia sinensis**) extract from dandruff causing microbes through antimicrobial activity.

OBJECTIVES

To estimate the tea leaves extract act as antibiotic on dandruff causing microbes *Malassizia globosa*, *M.restricta* sp

To find the species of *Malassizia* from the collected sample

To evaluate the growth of *Malassizia* species on SDA and PDA agar

CHAPTER 4 : MATERIAL AND METHODS

MATERIALS

1. Conical flask
2. Test tubes
3. Weighing balance
4. Autoclave
5. Centrifuge
6. Petri plates
7. Mesuring cylinder
8. Eppendorf
9. Laminar air flow
10. Glass slide
11. Pipette
12. Cotton swab
13. Beaker
- 14.** Inoculation loop

METHODOLOGY

Sample collection

A sharp and sterile epilation forceps was used to detach the hair and scalp samples from the infected persons. The infected region was first washed with 70% ethanol followed by scraping with the sterile epilation forceps which was held at an angle of 90° with the head. The specimen was then transferred into a dark sampling paper to prevent exposure to sunlight. Each sample was labeled with the patient's name. The samples were then taken to the laboratory for analysis.

Test organism

Malassezia globosa of clinical origin was used for the study. The cultures were subcultured and maintained on Sabouraud dextrose agar (SDA) slants and stored in refrigerator at 4°C.

Inoculum preparation

Inoculum of *M. globosa* was prepared by inoculating in 5 ml of Sabouraud dextrose broth and incubated at 30°C for 2 days.

Preparation of the extracts

Fresh leaves of tea leaves were collected and authenticated. All the samples were washed, dried in shade and powdered. The powdered sample tea leaves were separately extracted with , chloroform, ethyl acetate. Then the extract was filtered and concentrated on water-bath at low temperature. The final extract residue was re-dissolved in the respective solvent and made required Quantity

Culture

The sample collected were cultured on SDA (Saboraud Dextrose Agar)incorporated with chloramphenicol to rid the media of bacterial contaminants. Small amounts of the samples collected were introduced into Petri dishes containing the media using sterile forceps. The Petri dishes were then labelled and incubated at room temperature (25oC) for three days and then, up to a week .

Biochemical tests

Catalase test

Catalase test was carried out to ascertain the presence of *M. globosa* as it is, unlike *M. restricta* that is catalase negative, catalase positive. 3mls of 3% hydrogen peroxide (H₂O₂) solution was poured into a test tube. Several colonies of the isolated fungal colonies were immersed into the test tube using a sterile glass rod. Active bubbling observed, signifying the release of oxygen from the H₂O₂ indicated positive test for catalase production

Antifungal activity by 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. chloroform (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)

Identification

Direct microscopy

A drop of 10% potassium hydroxide, KOH with blue parker ink was introduced onto a slide containing the sample and covered with cover slip The purpose of applying the alkali was to digest the keratin surrounding the fungi so that the hyphae and conidia (spores) could be seen). The sample was then heated over a Bunsen flame to remove bubbles. The slides were viewed using x10 and x40 objectives with closed iris diaphragm of the lens of the condenser). Direct microscopy that showed the typical mixture of globose blastoconidia and pseudomycelia coupled with mycelia in the form of “sphagetti” indicated the most identifiable difference of *M. globosa* from the other species of *Malassezia* (Crespo

Gram's staining

A smear of the pure culture obtained after two weeks of incubation at 32°C and gram stained as demonstrated by This is with a view to studying the morphology of the yeast cells. A typical positive test is indicative of *M. globosa* .

Lactophenol cotton blue staining

1. On a clean microscopic glass slide, add a drop of 70% ethanol
- 2 . Add the fungal specimen to the drop of alcohol using a sterile mounter such as an inoculation loop (from solid medium), depending on the sample of use.
3. Tease the fungal sample of the alcohol using a needle mounter, to ensure the sample mixes well with the alcohol.
4. Using a dropper or pipette, add one or two drops of Lactophenol Cotton Blue Solution (prepared above) before the ethanol dries off.
5. Carefully cover the stain with a clean sterile coverslip without making air bubbles to the stain.
6. Examine the stain microscopically at 40X, to observe for fungal spores and other fungal structures.

Fungal spores, hyphae, and fruiting structures stain blue while the background stains pale blue.

Antifungal activity

sample	Zone of incubation
Control(chloroform)	nil
Chloroform(50 micro litter)	7m
Ethyle acetate(100micro litter)	2m

CHAPTER 5 : RESULT

The antifungal activity of tea leaves powder extract powder against *Malassezia globosa*, *M. restricta*. sample have collected from some persone dandruff. For this sample isolated SDA, PDA agar have been used. After the isolated it has been prepared for incubation on plate. on this process, the plate shoues cream like yellowish, smooth or lightly wrinkled colonies. by this identification we have isolated the cultured colonies and observe under the microscope the sample have stained by using lactophenol cotton blue and gram stain. microscopic appearance of stained sample have shown that it was *Malassezia globosa*, *M. restricta*. the result have show as figure on below



Figure1. Tea leaves powder



Figure2. Tea leaves extract



Figure3.SDA medium

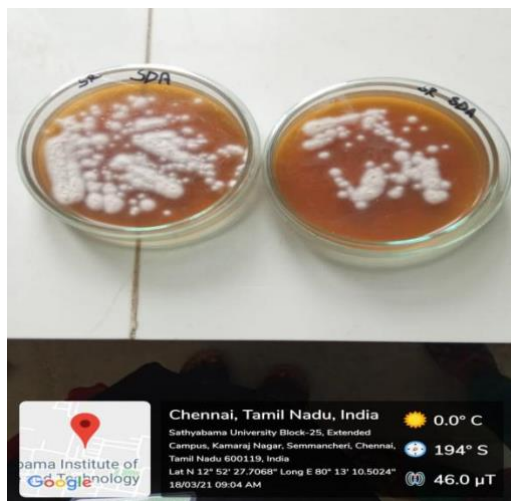


Figure4.Malassezia spp



Figure5.Antifungal activity

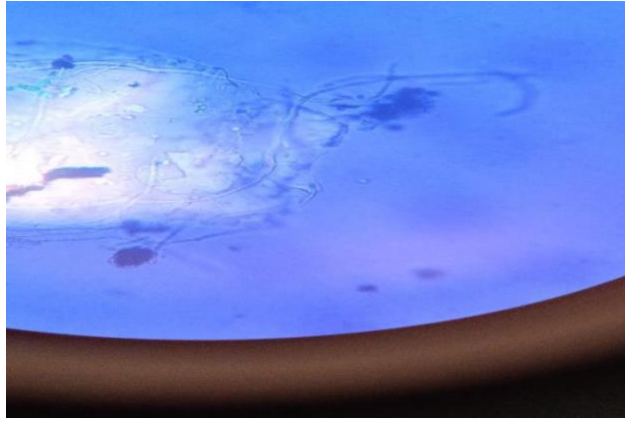


Figure6.Lactophenolcotton blue stain methods

CHAPTER 6: CONCLUSION

In effects of tea leaves in dandruff causing microbes. It was unexpected that live bacteria could be detected in the dry and long time store tea leaves.

Most of the identified bacteria belonging to the family basidiomycota, ustilaginomycotina, exobasidiomycetes, Malasseziales, Malasseziaceae and Malassezia have been found in human dandruff deep-sea vents and much everywhere in between. The skin of most if not all warm blood animals is covered with these microbes. However the degree between 60% and 70% rarely showed any microbial content. The tea leaves brewed still showed as relatively high bacterial count on SDA and PDA agar. The major bacteria *Malassezia globosa* and *restricta* were found in the tea leaves extract dandruff causing microbe. These isolates were also analyzed using disk diffusion method. The results showed that most of them have diffusion stain type. Based on the catalase reaction, direct microscopic morphology of the scalp and hair scraping as well as the microscopic morphology in gram stained smear the 150 specimens were identified as *M. globosa*, *M. restricta*.

Discussion

Dandruff is a common embarrassing scalp disorder affecting the people globally. The aim of dandruff treatment is to reduce the number of the *M. globosa* and *M. restricta* the scalp, and the goals of therapy are to reduce morbidity and prevent complications. 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-malassezial activity such as Ketoconazole, Zinc pyrithione, Octopirox, Climbazole and natural ingredients like Hyamarka (veppalai), Tea tree oil, Neem, Rosemary oil and Chamomile oil are commercially used in shampoos, lotions, creams and hair oils. However, these shampoos have to be used for a long period and leave scalp skin dry (which leads to frequent scalp irritation), and are also known to discolor light colored hair. Thus, with the available therapies, the problem of complete symptomatic control and clinical cure is usually not achieved and dandruff usually recurs on stopping these treatments. Plants contain innumerable active compounds which exhibit many diverse effects on biological systems. The neem tree has been used for centuries in India systems medicine and is sometimes referred to as the "Village Pharmacy".

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