IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

ISOLATION AND INVITRO CHARACTERIZATION OF HUMAN HAIR DANDRUFF CAUSING MICROORGANISMS

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Abstract: Human hair dandruff (HHD) from barber shop is a common unwanted scalp disorder that is prevalent to most human populations all over the world. Dandruff, a prevalent scalp condition, is often associated with microbial colonization on the scalp. Despite its common occurrence, the precise microorganisms responsible for dandruff formation remain unclear. This study aimed to isolate and characterize the microorganisms implicated in human hair dandruff through in vitro assays. The isolated strains were then subjected to various in vitro assays to elucidate their potential roles in dandruff pathogenesis. Through this comprehensive approach, we sought to deepen our understanding of the microbial composition and behavior associated with dandruff, providing valuable insights for developing targeted treatment strategies. This study was designed to isolate and characterize pathogens that are responsible for HHD as well as the evaluation of their biological control technique. Isolated bacteria were characterized by different biochemical tests and molecular identification methods. Here, Agar well diffusion methods were used to determine antibacterial activity against isolated bacteria. The isolated bacterial colonies were found to be Gram-positive, small, round-shaped, and purple. MALDI-TOF MS Test is done for the identification of microorganisms .In the antibacterial test, is done in *Phyla nodiflora* and *Wrightia tinctoria* identified zone of inhibition against the isolated bacteria. Moreover, from the plant oil extract phytochemical test is done. The present study would give a promising direction of identification and control of this pathogen biologically.

Keywords: Biological Control, Human hair dandruff (from barber shop),Microorganisms, Molecular detection, Plant extracted oil.

Introduction

Human hair dandruff (HHD) is a common disorder of the skin that affects the scalp and creates an unfavorable condition for most of the people over the world.Dandruff can contribute to several causes, including dry skin, seborrhea dermatitis, inadequate washing or scrubbing, too often shampoos, scalp psoriasis, eczema, hair-care or yeast-like fungus sensitivity(Ranganathan,Mukhopadhyay ,2010).Bacteria are more critical than fungi to the formation of dandruff, mostly Staphylococcus and Propionibacteria.The dominant fungus (Malassezia species) displayed contrary roles in its contribution to the healthy scalp micro-environment. Bacteria and fungi didn't show a close association with each other, but the intramembers were tightly linked. Bacteria had a stronger relationship with the severity of dandruff than fungi (Ro,B.I.&Dawson,et.al.,2005). Staphylococcus aureus is gram-positive and round shaped bacteria which are a component of the firmicutes and normal body fluids, mostly seen in the nose, breathing tract and the skin-(Tong SYC,et.al.,2015).To address this global issue, plants are considered to be a good source of traditional medicine, as are both bioactive and new therapeutic compounds. The plants are known since ancient times and widely accepted as a

crucial source of traditional medicinal compounds for specific diseases diagnosis.Therefore, this study focused on the investigation of bacterial isolation from a healthy volunteer with dandruff infected hair and treat with medici nal plants.

Materials and methods

Hair samples collection In present study, disease-associated hair samples were obtained from a healthy volunteer having a bad experience of this unpleasant scalp disorder who is not a member of our research group and willing to dedicate his unwanted hair scalp infected with dandruff as our research sample. Ethical clearance of the study was approved by the Director of the Institute of Biological Science, University of Rajshahi, Rajshahi-6205, Bangladesh (Approval no. IBSC.EC.5.6.18-00122).

Isolation and culture of bacteria

The hair sample collected in a sterilized zipper bag, and then hairs were taken in a conical flask that contained 100 ml distilled water. The flasks were shaken in a rotatory shaker for 30 minutes. One milliliter of water was taken into LB liquid medium by using a sterile pipette and incubated to grow bacteria at 37°C in a shaker overnight. On the next day, after incubation, a sterile loop was used to streak the bacteria onto a solid LB agar medium. The plated bacteria were cultured and incubated overnight, 16 hours, at 37°C.The pure culture was obtained through the streak-plate method. Finally, the plate was incubated overnight at 37°C. There were many single colonies found on the plates. To obtain the desired microorganism, sub-culturing was done, and the liquid medium was prepared for every single colony. The single colonies were taken by a loop of inoculation needle and touched on LB liquid medium on the laminar flow bench. Then it was incubated for 16-18h at 37°C temperatures.A loop of an inoculation needle was taken into the single colonies and touched on LB liquid media. It was then incubated at 37°C temperatures for 16-18 hours.

Biochemical characterization

The morphological and biochemical characteristics of the isolated bacteria were done. Bacteriological analysis was performed using selective media10-13. After 12 to 16 hours of growth in the LB Agar plate at 37°C, the morphology of the colony, size, shape, color, and growth patterns were recorded. Light microscopy was used to observe cell size. A series of biochemical tests namely, Gram staining, H2S production, Indole formation, Catalase, Simmon citrate, Methyl red, MSA test , Coagulase test, Oxidase test and Urease tests were performed to characterize the isolated bacteria based on Bergey's Systematic Bacteriology Manual guidelines14.

Molecular Detection for Malditof Test

The MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight) test for bacteria is a rapid and accurate method used for the identification of bacterial species. In this test, a small sample containing the bacteria is applied to a target plate and mixed with a matrix solution. When the sample dries, it forms crystals with the matrix.A laser beam is then directed onto the sample, causing the matrix to absorb the energy and vaporize, along with the bacteria. This process generates ions from the bacterial proteins. These ions are accelerated through an electric field and travel through a flight tube.The time it takes for the ions to reach the detector at the end of the flight tube is measured. This time, along with the mass-to-charge ratio of the ions, is used to create a mass spectrum unique to the bacterial species present in the sample.This spectrum is compared to a database of known bacterial species' spectra, allowing for rapid and accurate identification of the bacteria in the sample. MALDI-TOF is widely used in clinical microbiology laboratories due to its speed, accuracy, and ease of use.

Antibacterial Activity of Some Plant Oil Extracts

Two different varieties such as *Wrightia tinctoria* (vetpalai), *Phyla nodiflora* (poduthalai) were obtained from the village Veeranchimangalam Dharapuram road,Different plant parts were used as a potent source of plant oil extracts, described details,

Preparation of *Wrightia tinctoria oil* (vetpalai)

The leaves are collected in sufficient quantity. The leaves should be cleaned properly to remove the dirt and any other contamination.Once the leaves get dry, they are squashed with hands and put into the vessel.Then an equal quantity of coconut oil is added.The leaves are soaked in oil completely.Once this is done, the vessel is keptunder the sun in the daytime.



Fig 1 collection of plant Oil preparation Vetpalai oil

Preparation of *Phyla nodiflora* (poduthalai)

Wash the fresh poduthalai leaves thoroughly to remove any dirt. Chop the leaves into small pieces to facilitate the extraction process.Heat the coconut oil or chosen carrier oil in a saucepan over low heat.Add the chopped poduthalai leaves to the warm oil.Allow the mixture to simmer on low heat for about 1-2 hours, stirring occasionally. Once the oil has absorbed the essence of the poduthalai leaves and turned slightly green, remove it from the heat and let it cool down to room temperature. Strain the oil using a cheesecloth or fine mesh strainer to separate the leaves from the oil. Squeeze out as much oil as possible from the leaves.Transfer the strained poduthalai oil into a clean glass jar for storage.Store the oil in a cool, dry place away from direct sunlight.



Fig 2 collection of plant Oil preparation Poduthalai oil

Result:

Isolation of pure culture

After repeated microbial culture of the isolated pathogen in suitable medium and conditions described earlier, pure culture was found. The isolated colony was creamy white The colonies were small to medium, smooth, convex, and mucoid in size and shape. The colony morphology was identified by microscopic and visual observation.

Biochemical characterization

After isolation of the pure culture, various biochemical tests, including gram-staining (noted below) was done to characterize the isolated pathogen precisely.Gram-positive, appearing purple/blue under a microscope,Urease negative, meaning it does not produce urease and does not hydrolyze urea,H2S Production, does not produce hydrogen sulfide (H2S), so it is H2S negative.catalase positive, showing bubbles when hydrogen peroxide is added due to the presence of catalase enzyme. Indole negative, meaning it does not produce indole from tryptophan,Simmon Citrate,cannot utilize citrate as the sole carbon source, so it is citrate negative,MSA Test (Mannitol Salt Agar),ferments mannitol, causing the medium to turn yellow due to acid production, It can tolerate high salt concentration, growing well on MSA,coagulase positive, meaning it produces the enzyme coagulase, resulting in plasma clot formation,oxidase negative, meaning it does not produce the oxidase enzyme.These characteristics help in the identification and differentiation of *Staphylococcus aureus* from other bacteria.

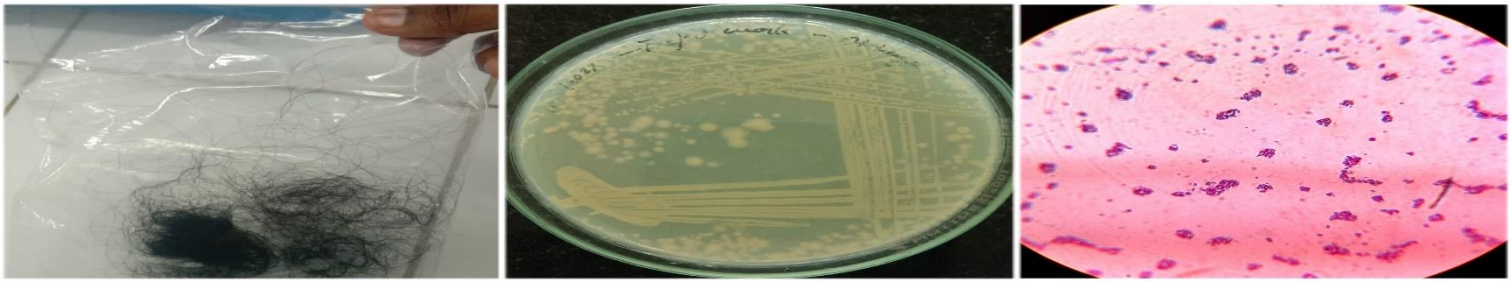


Fig. 3. Collection of hair samples, isolation of bacteria and gram staining of microorganism. (A) Hair sample (B) Isolated bacterial colonies and (C) Gram-positive bacteria

Table 1 .Biochemical characteristics of the isolated bacteria

Test	Result
Gram staining	+ve
Catalase	+ve
Indole test	-ve
Simmon citrate	+ve
Urease	-ve
Methyl red	-ve
H2S production	-ve
MSA	+ve
Coagulase test	+ve
Oxidase test	-ve

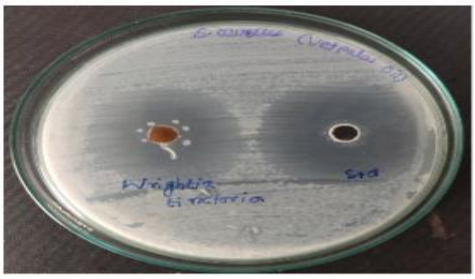
MALDI-TOF ANALYSIS:

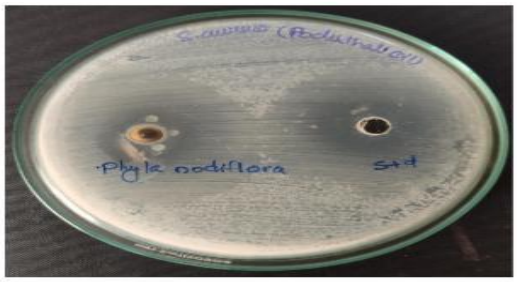
On the bacterial identification, the result was obtained as Gram-positive cocci and identified as *Staphylococcus aureus.*

Antibacterial Activity Against Isolated Bacteria by Some Plant Extracted Oil :

Well Diffusion Method:

In the provided image, the results of the antibacterial activity using the well diffusion method for vetpalai and poduthalai oils are displayed.Vetpalai Oil: The diameter of the zone of inhibition around the well containing vetpalai oil is measured as 1.2 cm.Poduthalai Oil: The diameter of the zone of inhibition around the well containing poduthalai oil is measured as 0.7 cm.These measurements indicate the extent to which each oil inhibited the growth of the tested bacteria. The standard reference values for comparison are also provided, with diameters of 1.5 cm for vetpalai oil and 1.0 cm for poduthalai oil. These values provide context for evaluating the effectiveness of the oils in inhibiting bacterial growth.





Organisms Concentration	*Vetpalai*	*Poduthalai*
Oil	1.2 cm	0.7 cm
Standard	1.5 cm	1.0 cm

Fig. 4. Antimicrobial activities against Staphylococcus aureus. (A) Wrightia tinctoria (vetpalai) ,(B)Phyla nodiflora (poduthalai)

Phytochemical Test:

The provided image shows the results of phytochemical analysis for vetpalai oil and poduthalai oil.Here are the observations for each test:Alkaloids: Vetpalai oil and poduthalai oil both exhibit reddish-brown precipitate (++).Flavonoids: Vetpalai oil shows a yellow color (+), while poduthalai oil shows a yellow color (+++).Phenol: Both vetpalai oil and poduthalai oil exhibit a brown color (++).Protein: Both vetpalai oil and poduthalai oil show a purple or violet color (+).Reducing Sugar: Vetpalai oil shows a blue color (+), while poduthalai oil shows a green color (+).Tannins: Vetpalai oil exhibits dark green or blue color (+), while poduthalai oil shows a greenish green color (+).Phytosterols: Both vetpalai oil and poduthalai oil show a blue color precipitate (++).Steroids: Vetpalai oil shows a red color precipitate (++), while poduthalai oil shows a blue color precipitate (++).Steroid Glycosides: Both vetpalai oil and poduthalai oil exhibit a red color or blue color (++).Saponin: Vetpalai oil shows foam formation (+), while poduthalai oil also shows foam formation (+).These observations provide insights into the presence of various phytochemical constituents in vetpalai and poduthalai oils, which may contribute to their biological activities, including antibacterial properties.

Test	Observation	Vetpalai oil	Poduthalai oil
Alkaloids	Reddish brown precipitate	++	++
flavonoids	Yellow colour	+	+
Phenol	brown colour	++	+++
Protein	Purple or violet colour	+	+
Reducing sugar	Blue colour	+	++
Tannins	Dark green or blue colour	++	++
Phyto sterols	Blueish green colour	+	+
Steroids	Red colour precipitate	++	+
Glycosides	Red precipitate	++	++
saponin	Foam formation	++	++

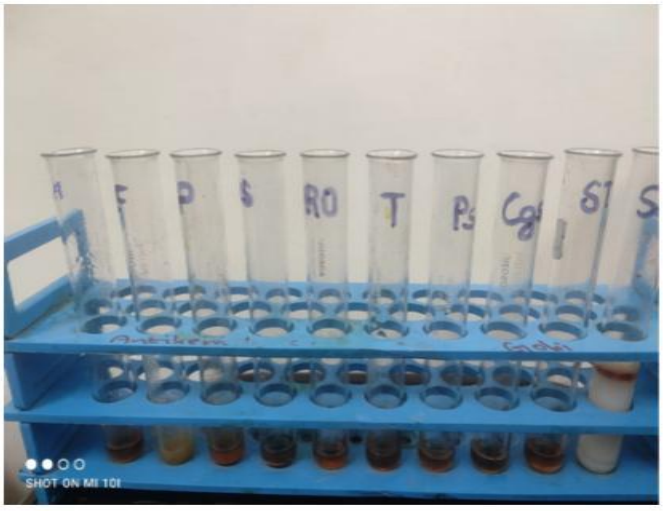


Fig .5. Phytochemical test against *Staphylococcus aureus (A)Wrightia tinctoria* (vetpalai) ,(B)*Phyla nodiflora* (poduthalai)

Conclusion

Human hair dandruff (HDD) is a common, unpleasant scalp disorder that is one of the major concerns for the health-conscious people over the world. In our recent work, we isolated dandruff, causing pathogens from a healthy

volunteer, experimented with some major biochemical and molecular characterization to control this disease using some globally available medicinal plants. In the Biochemical test, we proved that isolated bacteria as gram-positive and non-motile, glucose fermented, but lactose non-fermented.In molecular characterization, it confirms the isolated bacterial strain is Staphylococcus aureus that leads to identifying the associated genes or functional group for dandruff pathogenesis. In the antimicrobial test, *Staphylococcus aureus* shows the highest sensitivity to *Wrightia tinctoria* (vetpalai oil) among all the plant extracts that recommend a new prospect for developing an anti-dandruff compound. Although there were some limitations as we isolated pathogens from a single volunteer, the present study would be helpful for further direction of identification and control of this pathogen with biologically active compounds.

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