A STUDY OF ONYCHIA-RELATED MICROORGANISMS AND THEIR CONTROL WITH TRADITIONAL PLANT EXTRACTS AND EXUDATES

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ABSTRACT

In today's world, bacteria and fungi are the most common causes of nail infections. Onychia is a medical term that refers to an inflammatory reaction of the nail matrix, as well as the creation and removal of a finger or toe nail. In this study, we isolated and identified onychia-associated microbes, as well as their susceptibility to plant extracts and exudates. Biochemical methods were used to identify the isolates. Extracts from *Mangifera indica, Lawsonia inermis, Manikara zapota, Punica granatum,* and *Psidium guajava* exhibited significant effectiveness against Coagulase negative Staphylococci, *Pseudomonas aeruginosa, Candida albicans, S. aureus,* and Coagulase negative *Staphylococci*. However, the activity of these plants' exudates was limited to Coagulase negative *Staphylococci*, *Candida albicans,* and *S. aureus.*

INTRODUCTION

Currently nail infections are not unusualplace, which can be particularly due to each microorganism and fungi. Fungal contamination of nail is a not unusualplace contamination that influences one or greater nails of the hands and toes. It is due to microorganisms belonging to a collection of fungi, inclusive of dermatophytes, non-dermatophytes, and Candida. The inflamed nail progressively modifications in shadeation and chips away in pieces, or absolutely comes apart, because it will become brittle. Bacterial contamination is likewise not unusualplace. Bacterial paronychia contamination will seem suddenly, while fungal infections will take greater time. Bacteria can input

the frame via small wounds if they're now no longer well disinfected. The nail is a complicated unit composed of 5 most important changed cutaneous systems. The nail matrix, nail plate, nail bed, cuticle and nail folds. The cuticle is an outgrowth of the proximal fold and is located among the pores and skin of the digit and nail plate, fusing the systems together. This configuration gives a water evidence seal from outside irritants, allergens and pathogens.

Nail illnesses are wonderful from illnesses of the pores and skin. Although nails are a pores and skin appendage, they have got their personal symptoms and symptoms and signs which can also additionally relate to different scientific situations. Nail situations that display symptoms and symptoms of contamination or infection require scientific assistance. Diseases of the nail can be known as onychosis.

Onychia is used to outline a fitness hassle which is thought with the aid of using an inflammatory response of the nail matrix with improvement and lack of finger nail or toe nail. Onychia results from the induction of microscopic infectious agent for the duration of tiny injuries. This circumstance can also affect the nail mattress and therefore ensuing withinside the lack of the finger nail or toe nail. Onychia may be averted just like some other problem of this character, with the aid of using the usage of suitable hygiene.

In many instances the maximum probable cause in the back of onychia is a harm. The harm may be incurred at once or possibly because of misfitting footwear. Additionally, standard pores and skin fitness troubles as an example atopic dermatitis can also cause development of onychia. Diabetic problems are one greater standard cause for onychia as it performs a position in decreased blood float withinside the legs and reduced resistance to bacterial contamination. Cut withinside the dermis at the inspiration of the nail or likely if residing pores and skin is ripped away in preference to simplest the non-residing cuticle taken away, if so, that would make the matrix to be had to dangerous microorganism and capacity bacterial contamination. For the cause that the quilt guarding the matrix is damaged. The maximum not unusualplace reason of acute paronychia is direct or oblique trauma to the cuticle on nail fold. such trauma can be exceedingly minor, attributable to regular events, consisting of dishwashing, a harm from a splinter on thorn, onychophagia (nail biting), biting or selecting at a grasp nail, finger sucking, an ingrown nail, nail trimming procedures, synthetic nail application, or different nail manipulation. Such trauma permits bacterial inoculation of the nail and next contamination.

Paronychia is every other infection of the folds of tissues surrounding the nail of a toe or finger. Paronychia may be categorized as both acute or continual. The primary issue related to acute paronychia is direct or oblique trauma to article or nail fold. Paronychia has additionally been mentioned in affiliation with antiretro-viral remedy for HIV contamination, and with use of epidermal boom issue inhibitor. continual paronychia is a multifactorial inflammatory response of the proximal

nail fold to irritants and allergens. In continual paronychia, the cuticle separates from the nail flute, leaving the location among the proximal nail fold and the nail plate susceptible to contamination with the aid of using bacterial or fungal pathogens *Candida albicans* or microorganism can also additionally arise withinside the lesions.

The primary goal of the observe become to isolate and become aware of microorganisms related to onychia and their susceptibility to choose plants extracts and exudates.

2 MATERIALS AND METHODS

2.1 Collection of specimens

For the isolation of pathogenic microorganism, samples were collected from finger nail of patients with typical condition of onychia from nearby places (from college locations). Before taking samples, the patient's finger was washed with soap and wiped with alcohol. Gentle pressure is applied at the affected areas of nail and a sterile cotton swab was scrubbed on the finger nail of patients. After which the swab was put quickly into sterile peptone water and sealed. Then transported to the laboratory and incubated at 37^{O}C for 2-4 hours.

2.2 Isolation of pathogens

For the primary isolation of the pathogen's samples were streaked on different medias like Nutrient agar, Blood agar, Chocolate agar, MacConkey were used. To these medium, from the incubated peptone broth the organisms were inoculated by streaking. The plates were incubated at 37^{0}C for 24-48 hours. After incubation results were noted based on the morphological studies and gram staining reactions. Morphological characterisation was noted with respect to colour, shape, size and nature of colony.

2.3 Identification of the selected pathogens

For the identification of selected pathogens primary isolates were inoculated for various biochemical test (Indole test, Methyl red test, Voges proskauer test, Citrate utilization test, Catalase test, Coagulase test, Urease test and Triple sugar-iron (TSI) agar test) and also into various selective medias like Mannitol salt agar, king's B medium.

2.4 Antibiotic sensitivity test

To confirm the coagulase negative cocci as opportunistic pathogen, antibiotic sensitivity test was done. Inoculum of the test organisms were prepared in peptone water followed by incubation at 37^{o}C for 24 hrs. Lawn culture of the coagulase negative cocci were made by swabbing uniformly on surface of MHA. After swabbing the plates were allowed to dry. Antibiotic discs (penicillin G, Amoxycillin) were placed with a sterile forceps to ensure firm contact with the agar surface. Plates were incubated at 37^{0}C for 24hrs and, resistance or sensitivity of the organisms were noted.

2.5 Collection of plant materials

To check the susceptibility of selected pathogens against different plant extracts fresh plant leaves *Mangifera indica* (mango), *Lawsonia inermis* (henna), *Manikara zapota* (sapota), *Punica granatum* (promoganate), *Psidium guajava (*guava) were initially collected, cleaned, washed, shade dried for a total of 2 weeks, and then ground to a fine powder using a mortar and pestle. Plant exudates(latex) *Mangifera indica (*mango), *Carica papaya (*papaya), *Alstonia scholaris (*pala), *Artocarpus heterophillus* (jack fruit) were collected

2.6 Preparation of plant extracts.

10g of dried leaf powder previously made were weighed and mixed with 90ml distilled water in separate conical flask, covered with cotton plug and left for 24-48 hrs in a rotatory shaker at room temperature to get the maximum extraction. After 48 hrs the extracts were filtered off using filter paper (whattman no.1) in to a clean conical flask. After filtration it was poured into a petridish of known weight. The collected extract was allowed to dry at room temperature. The weight of the petridish containing the dried extract is weighed. The dried extract is dissolved in DMSO and stored under refrigerated condition for further study.

2.7 Antimicrobial and antifungal activity of selected pathogens

Lawn culture of the tested pathogens were made uniformly on the surface of Nutrient agar medium. After swabbing the plates were allowed to dry and wells were cut on the plates by using well borer. Wells of approximately 4mm in diameter were made on the surface of the solid medium and filled with 50 micro litres of the leaf extracts. Plates were incubated at 37^0C for 24 hrs and the diameter of zone of inhibition were measured in milli litre and tabulated in table4.

In similar way lawn culture were prepared for plant exudates and wells of approximately 4mm were made on the surface of the solid medium and filled with plant exudates. Plates were incubated at 37^0C for 24hrs and the diameter of zone of inhibition were measured in milli meter.

3 RESULTS AND DISCUSSION

Onychia is an inflammation of nail fold which can be due to infection in nail bed by wound or ingrown nail causing trauma which leads to pus formation, sometimes requiring surgical intervention like pus drainage or removal toe nail.

In this project an attempt was done to isolate and identify microorganism associated with onychia from nearby localities of our college and their susceptibility to selected traditional plant leaf extracts and exudates (latex).For the primary isolation of pathogens, the samples collected were inoculated into different media like Nutrient agar, Blood agar, Mac Conkey agar , Chocolate agar and incubated at 37^0C.The pathogens were isolated based on

their morphological characteristic , Gram's staining reaction and other differential properties on various media tabulated in Table 1 .

The primarily isolated pathogens were subjected to various biochemical reactions and selective tests to identify the pathogenic strains. Based on these tests, the pathogens were tentatively identified as coagulase negative *staphylococci* from sample A and sample E, *Pseudomonas aeruginosa* from sample B, *candida* spp. from sample C and *Staphylococcus aureus* from sample D. The results were tabulated in Table 2.

Table 1 Morphological Characteristics of primary isolates

Media colony characteristics	Sample A	Sample B	Sample C	Sample D	Sample E
Nutrient agar					
Colony size	small	large	small	Large	Large
Colony shape	circular	irregular	round	circular	Circular
Surface	smooth	Metallic sheen	Smooth, shiny	smooth	Smooth
Optical features	opaque	----	opaque	opaque	Opaque
Blood agar	Non haemolytic	Non haemolytic	haemolytic	haemolytic	Non haemolytic
Mac Conkey	Non lactose fermenting	Non lactose fermenting	Lactose fermenting	Non lactose fermenting	Non lactose fermenting

Table 2 Biochemical and selective identification of primary isolates

Biochemical tests	Sample A	Sample B	Sample D	Sample E
Indole	–	–	–	–
M R	+	–	+	+
V P	+	–	+	+
Citrate	–	+	–	–
Catalase	+	+	+	+
Coagulase	–	–	+	–
Urease	–	–	–	–
T S I	A/A	A/A	A/A	A/A
Motility	Non motile	motile	Non motile	Non motile
Mannitol salt agar	Non fermenting	Nil	Mannitol fermenting	Non fermenting
King's B medium	Nil	Typical green colonies	Nil	Nil

Sample C: germ tube technique (*Candida spp*) produces – short hyphae known as germ tube were observed

For the conformation of coagulase negative *staphylococci* as normal flora or opportunistic pathogen, antibiotic sensitivity test was done. It shows resistance to the selected antibiotic discs and tabulated in Table 3.

In the present a study, to check the antimicrobial activity of different traditional plant leaf extracts (*Mangifera indica, Lawsonia inermis, Psidium guajava, Punica granatum* and *Manikara zapota*) and exudates or latex (*Mangifera indica, Carica papaya, Artocarpus heterophyllus,* and *Alstonia scholaris)* were prepared. The leaf extracts showed a broad spectrum of antimicrobial activity with a zone of inhibition ranges from 0-25mm in diameter against different isolated pathogens.

Table 3 Antibiotic sensitivity test

Coagulase negative cocci	Name of antibiotic disk	observations	inference
Sample A	Amoxycillin, Penicillin	sensitive (2.2) sensitive (1.7)	Opportunistic pathogens
Sample E	Amoxycillin, Penicillin	sensitive (1.6) resistant	Opportunistic pathogens

The antimicrobial activity was done by using agar –well diffusion method.1% of the aqueous extract of different leaf extract in DMSO were prepared. 50 microlitre of extract were added into each well. The results were tabulated on Table 4.

Table 4 Antimicrobial activity of selected pathogens

Organisms	Extracts				
Coagulase negative *staphylococci*	*M. zapota*	L. *inermis*	*P. guajava*	*M. indica*	*P. granatum*
	14mm	15mm	17mm	13mm	17mm
P. aeruginosa	14mm	14mm	13mm	15mm	16mm

Candida albicans	10mm	14mm	15mm	11mm	13mm
S. aureus	12mm	15mm	14mm	12mm	25mm
Coagulase negative *staphylococci*	14mm	14mm	9mm	15mm	11mm

Among the different plant extract tested the maximum activity was shown by *Punica granatum* against *staphylococcus aureus.* The activity was around 25 mm and least was shown by Coagulase negative *Staphylococci* 11mm from sample E. Coagulse negative *Staphylococci* from sample A, *Pseudomonas aeruginosa* and *candida* spp shows almost similar activity with zone of inhibition ranges from 17mm,16mm,13mm respectively.

Among the *Psidium guajava* extracts the maximum activity was shown by coagulase negative *staphylococci* with zone of inhibition 17mm. All the other organisms show almost similar activity except coagulase negative *staphylococci* from sample E with 9mm. The maximum activity of *candida* spp was in guava extract 15mm.

While examining the activity of *Lawsonia inermis* extract, all the organisms including *Pseudomonas aeruginosa* shows the same activity with zone of inhibition ranges from 14-15mm.

By checking the activity of *Manikara zapota* extract coagulase negative *Staphylococci* and pseudomonas have maximum zone of inhibition, 14mm. while *Staphylococcus aureus* and *candida* species shows 12 and 10mm respectively.

By comparing the latex of different plants, *Carica papaya* shows activity against all the pathogens isolated except *Pseudomonas aeruginosa*. The maximum susceptibility by papaya latex was shown by *Staphylococcus aureus* 19mm and *Candida* by 15mm. While examining the susceptibility of *Alstonia scholaris*, *Pseudomonas aeruginosa* shows 18mm and *candida* spp shows no activity. Among the *Mangifera indica* latex the coagulase negative *Staphylococci* from sample A shows 16 mm and *Staphylococcus aeureus* 12mm zone of inhibition and among the *Artocarpus heterophyllus* latex it was 17mm and 16mm by pathogens (Table 5).

Table 5 Antimicrobial activity of latex of different plants

Exudates (Latex)				
	C. papaya	*M. indica*	*A. heterophillus*	*A. scholaris*
Coagulase negative *staphylococci*	12mm	16mm	17mm	12mm
P. aeruginosa	---	---	---	18mm
Candida Albicans	15mm	---	---	---
S. aureus	19mm	12mm	16mm	10mm
Coagulase negative *staphylococci*	16mm	---	---	---

Different plant extract and latex shows good activity against different pathogens with *Punica granatum* and *Carica papaya* as the best maximum. Plant extract possess various compounds like alkaloids, flavanoids, phenolic compounds tannin etc. which give antimicrobial property. *Punica granatum* contains many compounds like alkaloids, polyphenolics, ellayic acid and gallic acid which provides antimicrobial property. *Psidium guajava* leaves contains rich amount of einol, tannins, tri terpenes, flavanoids, resin, enyenol, malic acid,fat cellulose, mineral salt and a number of other substances. Whereas *Manikara zapota* leaves have antioxidant activity, the extracts contain phyto chemical compounds like phenols, reducing sugars, carbohydrates, favones, saponins, alkaloids, quinines and tannins. *Lawsonia inermis* contains mannite,tannic acid, and gallic acid, which are present in the form of mixture.

Alstonia scholaris latex contains phenolics including flavanoids and proanthocyanidins. *Carica papaya* contains many bioactive components including chymopapain and papain. Additionally, the plant also contains terpenoids, enyenol, thymol, saponins and alkaloids. *Mangifera indica* contains mangiferin a xanthone glycoside which is a major bioactive constituent, also mangiferin, tannins and gallic acid derivatives. Where as in *Artocarpus heterophyllus* compounds like morin, dihydromorin, cynomacurin, artocarpin, iso artocarpin, cyto artocarpin, artocarpesin, oxy dihydroartocarpesin and heterophylol are present.

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