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IDENTIFICATION AND NATURAL CONTROL MEASURES OF BACTERIAL ISOLATES FROM THE FOOT CRACK OF HUMAN BEING

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ABSTRACT

Cracks and fissures break the skin. Cracks are caused by result of skin that is too dry or too moist and over pressure. When skin is too dry, it can become rough and flaky. The warm moist environment between the toes particularly under little one is a perfect position for growth of some specialized bacteria, there is no evidence for bacterial identification. This study common pathological bacterial species, focused to identify responsible for the main cause foot crack. Identification of bacterial species as *Bacillus subtilis*, *Streptococcus pyogenes* and *Pseudomonas aeruginosa*. The CNSL (Cashew Nut Shell Liquid) has the great efficacy rate against bacterial species. It has the phenolic compounds and volatile oil. These bio-active compounds have significant activity against bacteria. Four different formulation of CNSL (25%, 50%, 75% and 100%) were prepared by dissolving with DMSO (Dimethyl sulphoxide). Anti-bacterial activity was studied on comparing all Petri plates. The present study revealed that, the 100% of CNSL showed the greater activity similar with standard drug Gentamycin rather than the diluted concentrations.

INTRODUCTION

The cashew (*Anacardium occidentale* .L), an evergreen species native to tropical America, can attain a 10-12m height. The species belongs to the class of the Magnoliophyta, order Sapindales and family Anacardiaceae. The cashew has been introduced and is a valuable cash crop in the America, West Indies, Madagascar, India and Malaysia. The commercial importance of cashew is due to its richness in nutrient that constituents of 47% fat, 21% protein and 22% carbohydrate, vitamins and all essential amino acids especially thiamine. The cashew nut consists of honey comb network of cells containing a viscous liquid called cashew nut shell liquid (CNSL). It is one of the sources of naturally occurring phenols and it obtained from the shell of a cashew nut. About 30-35% CNSL is present in the shell, which amounts to approximately 6% of the nut ^[1]. *Anacardium occidentale* of cashew containing the principal component phenols, Anacardic acid, cardol, 2-methyl cardol and cardanol ^[2].

Skin is a complex organ that requires the right amount of moisture to stay healthy. It can be hard to achieve the right balance of moisture in our feet. Moist skin can result from not drying feet after bathing from excess sweat, overly moist skin can encourage bacteria and fungus to grow ^[3]. Cracked heels are seen often in the elderly and females suffer more frequently than males. This dry condition of the skin is suitable place to bacterial growth and cause cracked heels. The problems worse during cold climatic condition. There cracks and fissures, severe as a portal of entry for bacteria. They are associated with an increased risk of cellulitis and foot ulceration that, if left unchecked, can eventually lead to amputation ^[4].

CNSL is obtained from the natural source and also free from hazardous material, it was chosen to the present study. Hence the study was designed to isolate and identify the bacterial species in the foot crack and also find the effective dose of cashew nut shell liquid against the pathogenic bacteria.

MATERIALS AND METHODS

Collection of Cashew nut:

The nuts of *Anacardium occidentale* .L were collected from cashew plantation of Udaiyarpalayam, Tamil Nadu. The nuts were collected in a sterile polythene bag by using sterile forceps.

Preparation of CNSL:

The cashew nut was cleaned and dried the shells were removed and cut in to small pieces. The liquid were extracted by heating to cashew nut shells (80°-100°C). The viscous liquid obtained from the cashew nut shell known as cashew nut shell liquid.

Oxidations of CNSL:

The CNSL was naturally acidic condition and Cashew nut shell liquid (2.5g) was dissolved in 10% aqueous sulfuric acid (100ml) and potassium dichromate (2.5g) was added and the mixture was stirred at 29°C for 24 hours ^[5]. There after DMSO solvent was dissolved in the CNSL four different concentrations 25%, 50%, 75% and 100% were prepared and used for the its antibacterial activity ^[6].

Isolation and Identification bacteria:

For the characterization of specific microbial species, the microbial sample was collected from the foot crack of human beings. 1gram of sample was ground well with 1ml of distilled water and make up to 10ml from this solution. The foot crack microorganisms were first isolated through serial dilution and identification of microbial species was performed using pour plate technique and with the biochemical tests ^[7].

BIOCHEMICAL ANALYSIS**Indole production test:**

Peptone broth was prepared by dissolving peptone (10.0g) and sodium chloride (5g) in 1000ml of distilled water and sterilized and inoculated with isolated organisms with a pH of 7.8. After 48 hours of incubation, 1ml of Kovac's reagent was added and allowed to stand for 10-15 minutes.

Methyl red test:

MR broth was prepared by adding peptone (7.0g), potassium phosphate (5.0g) and dextrose in 1000ml of distilled water with pH 6.9 and sterilized and inoculated with the test organisms and after incubation, 5 drops of methyl red indicator was added.

Vogas-Proskauer test:

VP broth was prepared by adding peptone (7.0g), potassium phosphate (5.0g) and dextrose in 1000ml of distilled water with pH 6.9 and sterilized and inoculated with the test organisms and after incubation, 5 drops of berri's reagent indicator was added.

Citrate utilization test:

Citrate agar medium was prepared by mixing ammonium dihydrogen phosphate and di potassium hydrogen phosphate (each 1.0g), sodium chloride (5.0g), sodium citrate (2.0g), magnesium sulphate (0.002g), bromothymol blue (0.08g) and agar (15.0g) in 1000ml of distilled water with pH adjusted to 6.8. The medium is then sterilized, inoculated with the test organisms and incubated at 37°C for 48 hours. After incubation slants were observed for growth and colour.

Tribble sugar indicate test:

TSI medium was prepared by mixing trypticase or peptone (10.0g), carbohydrates (5.0g), sodium chloride (15.0g), phenol powder (0.0178g) in 1000ml of distilled water with pH 7.3 and sterilized and inoculated with the test organisms at 35° C for 24-48 hours.

Urease production test:

The urea broth medium was prepared by adding urea (20.0g), yeast extract (0.1g), KH_2PO_4 (9.0g), K_2HPO_4 (9.5g), phenol red (0.01g) in 1000ml of distilled water with acidic range pH sterilized and inoculated the culture at 37°C for 48 hours.

Carbohydrate fermentation test:

Peptone water broth was prepared by mixing peptone (20.0g) in 1000ml of distilled water added. OF (Oxidation Fermentation) glucose agar medium was prepared by mixing peptone (20.0g), sodium chloride (5.0g), potassium phosphate (0.3g), bromothymol blue solution (15g), agar (3.0g) in 1000ml of distilled water and phenol red indicator was added. Insert an inverted Durgum tube in the culture tubes and incubate at 37°C for 48 hours.

Preparation of nutrient broth:

Stock cultures were maintained at 4°C on scopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of microorganisms from the test tubes of nutrient broth and incubated for 24 hours at 37°C, the culture were diluted with fresh nutrient broth.

Disc Preparations:

5mm (diameter) discs were prepared from Whatman No:1 filter. The discs were sterilized by autoclave at 121 ° C. After the sterilized the moisture discs were dried on hot air oven at 50° C ^[8]. The sterile discs were rinsed in CNSL at different concentrations (25%, 50%, 75% and 100%). These studies were performed in triplicate by using standard drug (10mcg/ Gentamycin). Control was used in dimethyl sulphoxide (DMSO) loaded 5mm sterile disc.

Antibacterial activity:

The disc diffusion method was used to screen the antibacterial activity ^[9]. *In vitro* antibacterial activity was screened by using nutrient agar. The nutrient agar plates were prepared by pouring 15ml of molten media in sterile petriplates. The plates were allowed to solidify and 0.1% inoculum suspension was swapped uniformly and the inoculums were allowed to dry for 5 minutes. The loaded disc was placed on the surface to diffuse for 5 minutes and the plates were kept for incubation, zone of inhibition formed around the disc were measured with transparent rulers in millimeters.

STATISTICAL ANALYSIS

Student's t-test was used for comparing two particular means. MS-EXCEL was used for computing the data and the statistical analysis.

RESULT AND DISCUSSION

In the present study, a wide range of human pathogenic bacteria were examined from the foot crack. Identified bacterial species are provided in the table I. It is based on their morphological and biochemical characteristics features. The bacterial species isolated from the foot crack of human being are as follows *Bacillus subtilis*, *Streptococcus pyogenes* and *Pseudomonas aureginosa*. The antibacterial activity of the CNSL was studied with reference to the standard drug are presented in table II. 25%, 50%, 75% and 100% concentration of CNSL discs were taken for the study. Among the four concentrations highest antibacterial activity was noted in 100% CNSL when compared with 25%, 50% and 75% of CNSL. Maximum antibacterial activity was observed in *Streptococcus pyogenes* (5.26±0.26, 8.98±0.51, 12.04±0.60, 19.9±1.24 mm in diameter respectively) and *Pseudomonas aeruginosa* (4.74±0.44, 10.78±0.62, 12.94±1.70, 19.88±1.91 mm in diameter respectively) and *Bacillus subtilis* (4.12±0.11, 6.84±0.45, 10.46±1.11, 19.8±1.61 mm in diameter respectively). When compared with foot crack bacteria and the standard anti- diabetic (Gentamycin). Comparison of antibacterial activity of CNSL, Standard and Control are presented in Figure I. The absence of zone of inhibition around each well signified resistance. It was observed that the extract (DMSO) used as control did not inhibit the growth of the test bacteria.

Crude extracts from eight Nigerian medicinal plants used traditionally in the treatment of infections and specific diseases in both humans and animals were screened *invitro* for antibacterial activity using the hole plants *Angeiossus schimperi* and *Anacardium occidentale* which gram negative bacteria^[10].

The activity of the fractions may be attributed to the presence and action of the phytochemicals. The antibacterial activity of leaf extracts of *Eucalyptus camaldulensis* in which it was reported that the activity of the extracts was due to the presence of the phytochemicals especially polyphenolic compounds and volatile oils^[11]. The mechanism of action of tannins is based on their ability to bind proteins thereby inhibiting protein synthesis^[10]. The extract of *A. occidentale* exhibited *in vitro* antimicrobial activity against 13 of 15 microbes examined. In the same study, the extracts of *A.occidentale* leaf was reported to exhibit activity against gram negative bacterium *Helicobacter pylori* which are a causative agent of stomach ulcer^[12]. The methanol extract of the leaves of *A. occidentale* was more active against all test bacteria than the aqueous extract. This

may be due to the ability of methanol to extract a wider range of antibacterial principles than the aqueous solvent ^[13]. The leaf methanol extract was also observed to be more active than the stem bark extract. The phytochemical analysis of the stem bark of *A. occidentale* revealed the presence of alkaloids tannins, alkaloids, saponins, terpenes and flavonoids in *A. occidentale* leaves ^[14]. Thus the antimicrobial activity of the extracts on the test organisms may be due to the presence of the above phytochemical components.

The CNSL liquid obtained from the shell of the nut, cashew nut shell liquid (CNSL) have wide commercial applications ^[15-17], biological and medicinal properties. The biological properties of CNSL such as larvicidal ^[18], molluscicidal ^[19-20], antifungal and anti-microbial ^[21-22], were also reported. The medicinal properties of phytochemicals present in CNSL reported are cytotoxic activity against several tumor cell lines ^[23], anti-diabetic ^[24], anti-inflammatory and analgesic effects ^[25-26].

CONCLUSION

The cashew nut shell liquid has high economic value in different ways. The neutralization of crude extracts of CNSL does not contain harmful substances having more advantages than the commercial available drugs. It does not create any side effect, so it was promising agent to control the bacterial species of foot cracks, so it was advised to apply CNSL twice a day. Further better management of foot crack and fissures. The recent findings demonstrate that CNSL helps in healing of cracks, reducing scaling and pain. It is useful in making feet soft and smooth. None of the volunteers experienced any hypersensitivity reactions. Therefore it may be concluded that the feet medication with CNSL is can efficient management of foot cracks.

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Table: I. Morphological and biochemical characterization of Isolated Bacteria

Characteristics	<i>B. subtilis</i>	<i>S. pyogenes</i>	<i>P. aeruginosa</i>
Morphological Features			
Cell shape	Rod	Cocci	Rod
Gram stain	Positive	Positive	Negative
Motility	Motile	Non motile	Motile
Colony colour	White	White	Blue-green
Colony shape	Round	Round	Round
Bio chemical tests			
Indole production test	-	-	-
Methyl red test	-	+	-
Voges-Proskauer test	+	-	-
Citrate	+	-	-
Triple sugar Indicate	A/A +	K/K -	A/K ±
Urease	-	-	-
Carbohydrate fermentation	+	+	+
a.Glucose	+	+	+
b.Sucrose	+	+	+
c.Lactose	+	+	+

A-Acid (+), K-Alkaline (-)

A/A A- acid butt, A-acid slant

K/K K-alkaline butt, K- alkaline slant

Table: II. Antibacterial activity for different concentration of CNSL.

Test micro organisms	Zone of inhibition (mm in diameter)					
	25%	50%	75%	100%	Standard*	Control
<i>Bacillus Subtilis</i>	4.12±0.1116	6.84±0.4586	10.46±1.1110	19.8±1.1661	23	R
<i>Streptococcus pyogenes</i>	5.26±0.2653	8.98±0.5192	12.04±0.6829	19.9±1.2433	25	R
<i>Pseudomonas aeruginosa</i>	4.74±0.4498	10.78±0.6241	12.94±1.7012	19.88±1.9177	27	R

*Standard drug - Gentamycin.

C – Control DMSO.

R- Resistant to Inhibition.

Figure I: Comparison of antibacterial activity of CNSL, Standard and Control