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Extraction And Monitoring Antimicrobial Activity, Dehydrogenase Activity And Pesticidal Activity Of Cashew Nut Shell Oil (Cnsl)

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Abstract: CNSL is a by-product of *Anacardium Occidentale. L*. A mixture of cardanol, cardol, anacardic acid and methyl cardol, is a versatile by-product of the cashew industry. CNSL contains approximately 60-65% anacardic acid, 15-20% cardol, 10% cardanol, and traces of methyl cardol. The oil has been found to have potent antimicrobial properties. The evaluation of antimicrobial activity, dehydrogenase activity and pesticidal activity of the extract was obtained in the study. The antimicrobial activity was evaluated by agar well diffusion method and it was determined against *Staphylococcus aureus, Streptococcus agalactiae, E.coli, Pseudomonas aeruginosa, Candida albicans and Aspergillus niger*. The extracts were compared with standard disc of ampicillin. Determination of dehydrogenase enzyme activity in the soil samples gives us large amount of information about biological characteristic of soil which is essential for maintaining soil fertility as well as soil health, by adding CNSL to the soil the effect of dehydrogenase activity on soil is determined. The pesticidal activity of CNSL is also evaluated in this study.

Index Terms- Antimicrobial Activity, Cashew Nut Shell Oil, Dehydrogenase Activity

I. INTRODUCTION

Since ancient times, people have sought medicine for relief and recovery from illness. Ancient scriptures such as, Indian Holy book Vedas, Chinese book on roots and grasses, The Holy Bible and preserved monuments have evidence that humans have been trying to study medicinal plants for a very long time. The isolation and identification of biologically active compounds and molecules from nature have led to the discovery of new therapeutics and improvements in the healthcare and pharmaceutical sectors. Knowledge of ethno-botany is of great importance because it deals with the relationship between plants and animals and opens the door to the discovery of new potential bioactive compounds from plant species. The World Health Organization (WHO) has noted that most of the people use medicinal plants for the primary treatment, thus making recommendations to encourage countries to develop national policies and regulations on the use of traditional medicines with proven effectiveness.

The cashew tree (*Anacardium Occidental L*.) is a medium-sized tropical tree commonly grown for its fruit (cashew nuts) and pseudo-fruit (cashew apples). The homeland of cashew tree is Brazil, but it came to India four centuries ago from Portuguese travelers and took deep roots in India. In India, it is cultivated in Malabar, Kerala, Karnataka, Andhra Pradesh, Tamil Nadu, Goa, Orissa, Maharashtra and West Bengal. Cashew nut shell liquid is a major by-product of cashew processing due to its unique chemical properties making it an excellent source of unsaturated long-chain phenols and highly resistant acids and alkalis. Other properties, such as resistance to termites and insects, make these resins suitable for agricultural applications, while their antibacterial properties expand their use in the medical field.

1.2 Morphology

The tree can grow up to the 6-12 meters. Leaves are spirally arranged and elliptical in shape. The true fruit is nut which is double-walled shell with a hard epicarp, a poisonous mesocarp and a thin endocarp. The edible part of the fruit is kernel with a testa and pseudo fruit that is called the cashew apple is pear or apple-shaped and it is edible.

II. Statement of the Problem

The most important product of the cashew tree is the nut, which is used to make confectionery. Cashew nut liquid (CNSL) had great industrial importance which is obtained from the seed coat by distillation or steam or heat extraction. Cashews are a valuable edible nut that produces two types of "oils", one of which is found between the seed coat or nut shell and the nuts. After taking out edible part the outer shell is a waste product. Thus it can be used to make efficient products from it, as it has different properties which could be helpful for humans and plants. The chemical pesticides and fertilizers are not environmental friendly and they cause serious effect on humans and environment, a country like India is mostly dependable on agriculture for livelihood thus it is important to promote the research on eco friendly practices in agriculture. Therefore**, t**his work intends to study the antimicrobial properties and dehydrogenase effects and pesticidal activity of CNSL.

III Aim and Objectives of the study

The aim of this research is to investigate the extraction of cashew nuts and identifying antimicrobial properties, dehydrogenase activity and pesticidal activity.

Therefore, the specific objectives are as follows;

1. To examine the features of cashew nut tree and cashew nut shell oil.
2. To extract oil from cashew nut shell.
3. To determine the antimicrobial properties of the cashew nut shell oil.
4. To determine the dehydrogenase activity of the cashew nut shell oil.
5. To determine the pesticidal activity of the cashew nut shell oil.

IV Review of literature

Shabeeba M Ashraf *et al*. conducted a study on Antibacterial and anticancer activity of the purified cashew nut shell liquid: implications in cancer chemotherapy and wound healing (2018). The LC-MS data revealed that the CNSL contains the cardanol, anacardic corrosive and methylcardol. It restrained the expansion of HeLa cells with an IC50 of 0.004 %(v/v) and caused moderate mitotic block with shaft irregularity. It prompted apoptosis in HeLa cells and sped up injury conclusion in L929 cells. It hindered the growth of Bacillus subtilis with an IC50 of 0.35%(v/v) and the treated cells showed extended morphology demonstrating that suppression of cell division is one of the potential components of its action. The study suggests that the purified CNSL might have potential applications in anticancer and antibacterial drug development.

Yuttana Sudjaroen *et al* investigated Antioxidant, Antibacterial and cytotoxicity activities of cashew nut shell waste. The study proved that cashew nut shell waste extract (150µg/ml) inhibited DPPH and ABTS radical by 75.5 ± 1.4 and 97.1 ± 1.4%, which calculated to 57.1 ± 1.0 and 56.2 ± 0.6µmol TEAC, respectively. The study concluded that antibacterial activity of CNSL or cashew nut shell waste was affected to Gram-positive bacteria.

Vasanth pandian *et al* studied the use of Cashew Nut Shell Liquid (CNSL) in bio-pesticide and bio-fertilizer. The study proved that CNSL has an application not only as biofertilizer but also as biopesticide for the control of pest and insects and they offer potential benefits when used as substrates in developing bioprocesses for the production of organic chemicals and bio-molecules

According to the study conducted by Agniezka wolinska and Zofia stepniewska on Dehydrogenase activity in the soil environment Soil is a part of the terrestrial compartment, and supports all terrestrial life forms

V Methodology

Materials Required

Bunsen burner, Muller Hinton Agar , Microbial culture (*S. agalactiae, S.aureus, E.coli, P.aerugnosa ,Candida albicans, Aspergillus niger),* Sterile cotton swabs , Petri dishes, Cork borer / micropipette tip , Sterile teasing needle, Melted Muller Hinton agar , Micropipette , CNSL, Positive control disc , TTC solution ,Glucose solution , Screw cap tube , Distilled water , Soil , Methanol ,Pseudomonas agar base , Sabourdox dextrose agar , Nutrient agar , Mannitol salt agar , Eosin methylene blue agar.

Isolation of Microorganisms

Soil sample collection

The soil sample is collected from the IRTC garden using a sterile spatula. A 'V' Shaped cut is made and soil is taken from 15 cm depth. The collected soil is stored in a sterile petriplate.

BACTERIA

For *pseudomonas*sp

Soil dilutions is made by suspending 1 gm of soil in 9ml.10 $^{-5}$,10^{-6},10^{-7} were taken for isolating *pseudomonas spp*. 1ml of the sample from respective dilutions is pipettedinto the pseudomonas agar base medium. The plates were gently rotated, solidified and incubated for 24 hours at 37°c.

For S.*aureus*

Mannitol salt agar plates were prepared .The skin scalping was directly placed in petriplate with the help of sterile slides. Then it is kept for incubation at 37°c for 24 hours. From the grown culture pure culture is performed.

For *e.coli*

The water sample from bore well was collected .The sample was then transferred into EMB broth and incubated at 37°c for 24 hours. The sample was then inoculated into sterile EMB agar plates and incubated at 37°c for 24 hours.

For *streptococcus agalactiae*

The sample from wound is taken and inoculated in blood agar plate and incubated for 24 hours and it is inoculated into nutrient agar plate and incubated for 24 hours at 37°c.

FUNGI

Soil plate method:

Soil dilutions were made by suspending 1g of soil of each sample in 9ml of sterile distilled water. Dilutions of 10^{-3}, 10^{-4} and 10^{-5} were used to isolate fungi in order to avoid over-crowding of the fungal colonies. 1ml of the suspension of each concentration was added tosterile Petri dishes, in triplicates of each dilution, containing sterile Sabourdox Dextrose Agar medium .The plates were then incubated at 28°c for 4-7 days.

Sample collection

The cashew nuts from the cashew farmers of Mundur, Palakkad district, Kerala were collected.

Pretreatment of samples

Some basic physical operations were carried out on the samples (cashew nuts) before extraction to ensure high degree of purity and quality of product. These operations include washing; drying .The nuts were first washed thoroughly and rinsed subsequently with distilled water after removing the fruit part so as to remove any dirt and contaminant that might have been attached with it.

EXTRACTION OF CASHEWNUT SHELL OIL

figure 5.1

figure 5.2

Roasting pan is an open circular dish and heated over an open fire. 1kg of raw nut is placed on the heated pan. As the nuts heat up, the CNSL is exuded into the pan and eventually ignites producing cloud of thick black smoke. The extracted oil is collected and stored in an sterilized air tight container.

MONITORING ANTIMICROBIAL ACTIVITY

For Bacteria

The agar surface plate is inoculated by spreading a volume of microbial inoculum over the entire agar surface by lawn culture. To the MHA around 6-8 mm diameter wells were made. The wells are punctured with the back side of a micropipette tip. Then CNSL of different dilutions that is 75ul, 100ul and 125ul added to the well using micropipette and MH broth to the control well .Add the positive control strip to the other side of the disc using sterile forceps. The plates were incubated at 35° to 37° for overnight in an upright position. After incubation looked for zone of inhibition and measured the zone size and compared with control disc.

For Fungi

The SDA plates were prepared and the wells are punctured with the backside of a micropipette tip. *Candida albicans* and *Aspergillus niger* is inoculated by using sterile inoculation needle. The CNSL is added to the wells of dilutions 75ul, 100ul and 125ul. The plates are inoculated for 3-4 days and after incubation look for zone of inhibition and measure the zone size.

DEHYDROGENASE ACTIVITY OF CASHEWNUT SHELL OIL IN SOIL

1gm of air-dried soil was taken in an air-tight Screw capped test tube. 0.2 ml of 3% TTC solution is added in each of the tubes to saturate the soil. 0.5 ml of 1% glucose solution is added in each tube. The bottom of the tube is tapped to drive all trapped oxygen. A water seal was formed above the soil. Ensured no air bubbles were formed. The tubes are incubated at 28 +0.5 C temperature for 24 hours. After incubation 10 ml of methanol is added. The tubes were vigorously shaken and allowed to stand for 6 hours. The clear pink colored supernatant liquid is withdrawn and the absorbance at a wavelength of 485 nm (blue filter) is read.

PESTICIDAL ACTIVITY OF CASHEW NUT SHELL OIL.

The extraction is applied to the pest as contact pesticide. The collected pests were taken in sterilized Petri dish. With the help of dropper the extraction is applied to the pest and the time taken to kill the pests is observed. Insects in capable of moving after a slight touch were considered as dead.

Collected Pests

1. **Black bean aphid**
   Scientific name: *Aphis gossypii*
   Order: True bugs
   Higher classification: Aphis
   Family: Aphididae
   Rank: Species
   Class: Insecta

2. **Spiralling white fly**
   Scientific name: *Aleurodicus dispersus*

   Family: Aleyrodidae

   Order: True bugs

   Species: A. dispersus

   Genus: Aleurodicus

   Phylum: Arthropoda

   Rank: Species

3. **Guava moth**

   Scientific name: *Argyresthia eugeniella*

   Phylum: Arthropoda

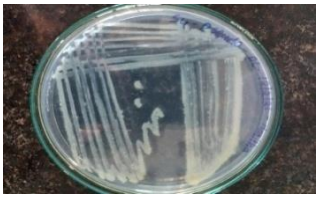
   Rank: Species

   Order: Lepidoptera

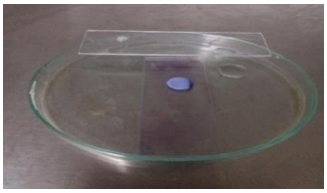
VI RESULT AND DISCUSSION

Extraction of cashew nut shell oil

The oil is collected and stored in sterilized air tight container for further use.



Isolation of Microorganisms



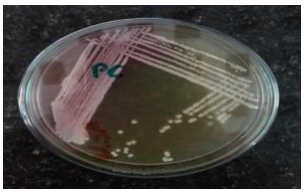
Bacteria

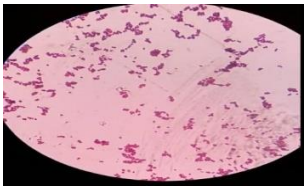
Pseudomonas spp.

figure 6.1 colony growths for *Pseudomonas* is observed from pseudomonas agar base medium

figure 6.2 pure culture plate is prepared by quadrant streaking method.

figure 6.3 oxidase and catalase test positive



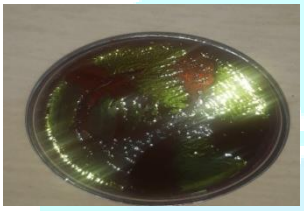


S.aureus

figure 6.4 pure culture plate.

figure 6.5 on gram staining purple colored, spherical shaped, grape like cluster morphology is identified.

E.coli



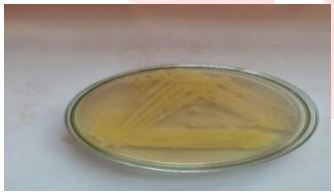


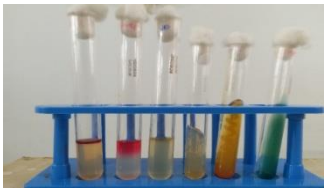
figure 6.6 Pure culture plate of *E.coli*

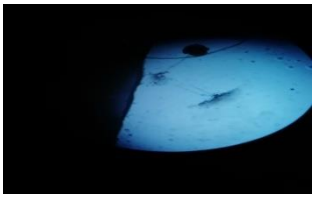
figure 6.7 Biochemical test result

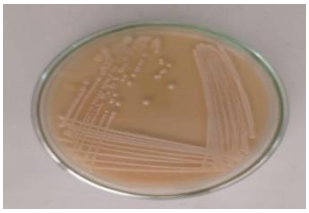
Streptococcusagalactiae

figure 6.8 culture plate

figure 6.9 biochemical test result







FUNGI

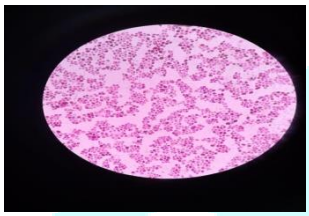
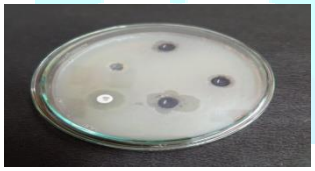


figure 6.10 LPCB technique it is identified as *Aspergillus niger*.



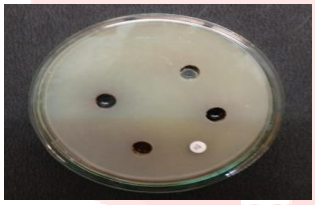


figure 6.11 culture plate of candida albicans

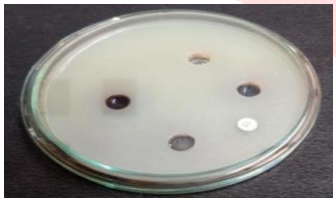


figure 6.12 Gram staining result of *Candida albicans*

Antimicrobial Activity

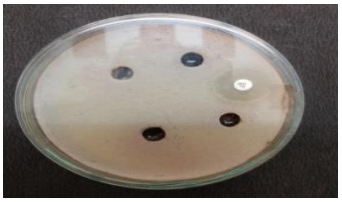


figure6.13 *S.aureus* showed inhibition against cashewnut shell oil. The dilution of 125ul showed highest inhibition of 1cm.The dilution 100ul showed 0.4cm inhibition zone.The75ul showed 0.1cm inhibition zone.

figure 6.14 *Streptococcus agalactiae* showed resistance.

Figure 6.15 E.coli showed resistance.

figure 6.16 The *Pseudomonas aeruginosa* showedresistance

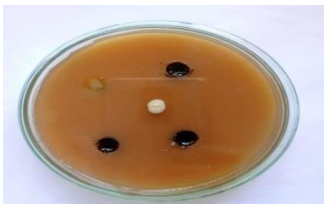


figure 6.17 *Candida albicans* showed no zone of inhibition.

figure 6.18 Aspergillus showed no zone of inhibition

Dehydrogenase Activity

Formation of Tpf

figure 6.2.1

Dehydrogenase Activity Table

Soil sample	285nm
100ul	91nm
200ul	74nm
300ul	43nm

Table 6.1- Dehydrogenase activity of different dilutions. The wavelength is decreasing while the dilution increases.

Pesticidal Activity

PEST	**TIME TAKEN FOR KILLING**
Black bean aphid	5 minutes
Spiraling white fly	8 minutes
Guava moth	5 minutes

Table 6.2- Pest name and their time taken to kill after spraying extraction.

figure 6.2.1 picture showing after spraying of CNSL on black bean aphid

figure 6.2.2 Pictures showing before and after spraying CNSL on spiraling white fly.




figure 5.2.3 pictures showing before and after spraying of CNSL on guava moth.

VII RESULT AND DISCUSSION

The oil is extracted using open pan roasting method and the secreted oil is collected in an airtight container. The Microorganisms where isolated from soil and water sources. The colony growth of p*seudomonas sp*, *Staphylococcus aureus*, *E.coli*, *Streptococcus agalactiae*, identified in Psuedomonas base agar medium, MSA medium, EMB agar medium and nutrient agar medium respectively. The fungal colony is identified in Sabourdox Dextrose Agar medium. The *S.aureus* showed inhibition against cashew nut shell oil. The dilution of 125ul showed highest inhibition of 1cm.The dilution 100ul showed 0.4cm inhibition zone. The 75ul showed 0.1cm inhibition zone and others resisted CNSL oil. The highest zone formed is 1cm and shortest is 0.1cm. The activity of dehydrogenase is used to identify the effects of CNSL on soil quality and fertility. DHA of soil was determined by using triphenyltetrazolium chloride as hydrogen acceptor, which was reduced to form red formazan. CNSL dehydrogenase activity of different dilutions is performed. The amount of formazan produced in 1gm of dry soil for 6 h was an active unit of dehydrogenase. Colorimeter is used to identify the microbial activity. When the dilution rate increases, the wavelength is decreasing thus we can assume that nutrient availability of soil is increased. The pests black bean aphid, spiraling white fly and guava moth showed fatal in 5, 8, 5 minutes respectively.

VIII CONCLUSION

This study revealed that CNSL has great potential for therapeutic treatment of antimicrobial activity against *Staphylococcus aureus*. The other microorganism doesn't show any zone of inhibition there for concluded as it is resistance against CNSL. The presences of various bioactive components in cashew nut shell extract have been attributed to its potent antibacterial activity. However, Pharmacological investigation should be performed by using advanced technique to discover the potential of the cashew nut shell extract. The soil DHA is one of the effective biological indicator for analyzing the soil quality and microbial activity. The result on impacts of CNSL on soil has proved the fertility of soil was improved by microbial activity. The CNSL acted as a phytopesticide for the eradication of one of the native plants. Thus the study proved CNSL has an application as a bio-pesticide for the control of insects and pests. From economic and environment point of view using plant based products are much more beneficial and it aids in continued discovery of novel drugs.

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