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A FIELD PROJECT REPORT ON

Organic food supplement for skin

Submitted in fulfilment of the requirements for the award of the degree of

BACHELOR TECHNOLOGY

In

BIOTECHNOLOGY

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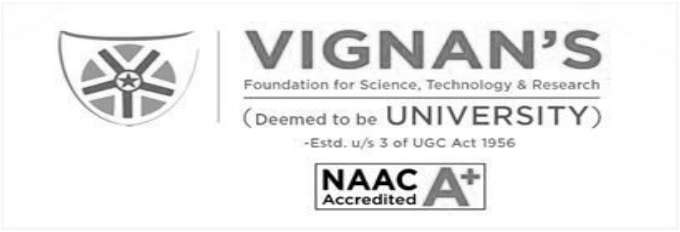

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December-2023

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CERTIFICATE

This is to certify that the field project entitled "**Organic food supplement for skin**" being submitted by Mandalapu Geethanjali (211FA14048), Cherukuri Madhusree (211FA14049), Naidu Likhitha Priya (211FA14050), Hari Priya Narra (211FA14051) in the partial fulfilment of Bachelor of Technology project in the department of BIOTECHNOLOGY, Vignan's Foundation for the science technology and Research, Vadlamudi, Guntur District, Andhra Pradesh, India, is a bonafide work carried out by them under our guidance and supervision.

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Prof. T.C. Venkateswarulu
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DECLARATION

We hereby declare that our project work described in the field project titled **Organic food supplement for skin**" which is being submitted by us for the partial fulfilment of the internet of things project in the department of Biotechnology, Vignan's Foundation for Science, Technology and Research (Deemed to be University), Vadlamudi, Guntur, Andhra Pradesh, and the result of investigations are carried out by us under the guidance Dr.A.Vijayasai

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CONTENTS

1) INTRODUCTION

Nowadays, consumers are driving innovation in cosmetics through the use of safe ingredients. They have increasingly preferred cosmetics made of natural ingredients with active functions on their skin and would prefer to pay more for a cosmetic that promises more skin benefits (Draelos, 2019). Food-derived ingredients have been explored to obtain "healthier" cosmetics (Anunciato & da Rocha Filho, 2012).

The concept of "Cosmeceutical" was first described in 1962 by Raymond Reed (Reed, 1962) and spread in 1980s by Albert Kligman (Kligman, 2006) based on scientific research of tretinoin administration for ultraviolet radiation (UV)-damaged skin (Feetham, Jeong, McKesey, Wickless, & Jacobe, 2018). Cosmeceuticals are topical products with effects on both skin appearance and functioning. These products are more than a simple cosmetic, being a mixture of "cosmetic" and "pharmaceutical". Scientifically, cosmeceuticals have similar properties to pharmaceuticals, and thus, have a lasting effect through a physiological and/or pharmacological action (Anunciato & da Rocha Filho, 2012; Feetham et al., 2018; Kligman, 2006; Reed, 1962).

Other concepts have appeared along time as "Nutraceuticals" and "Nutricosmetics" (Fig. 1). Nutraceuticals were defined by DeFelice et al. (DeFelice, 1995), as "any food or part of a food that provides medical or health benefits". This concept started to gain more attention in the 80s with the discussion of the food components benefits and the role on human health of each active component (Anunciato & da Rocha Filho, 2012; DeFelice, 1995). These active agents were regarded as key elements in the skin protection from damage and antiageing effects (Pearson, 2018).

Nutricosmetics can be described "as the consumption of food or oral supplements to produce an appearance benefit and are also called ''beauty pills,'' ''beauty from within,'' and even ''oral cosmetics'' (Anunciato & da Rocha Filho, 2012; Pearson, 2018).

All these concepts have arisen due the perception that we are surrounded of food capable of having effects in human body including skin. For example, fruits and vegetables have many variable antioxidants (Lademann, Meinke, Sterry, & Darvin, 2011).

More recently, several research groups have started to process plant and food waste to obtain new sources of active agents, mostly antioxidants, antimicrobials and antiageing compounds to be incorporated in cosmetic products. This has led to the selective extraction of mainly phytochemicals from natural material, such as vegetables, creating the opportunity to substitute synthetic chemicals that are currently in use in cosmetic industry (Fig. 2) (Peixoto et al., 2018). This is a growing market that promises to improve our general health as well as ecological recycling issues (Taeymans, Clarys, &

Barel, 2014, pp. 583–596). In fact, efficient and low-cost re-use of industrial by-products for high added-value products in the context of health and well-being represents a real societal challenge from the technological, economic and ecological point of view.

1. PROBLEM STATEMENT

An experimental study of the rhizosphere effect on phytoremediation of petroleum polluted soil was carried out with three species of grasses, namely Pannicum, Eleusine indica (L.) Gaerth, and Tall Fescue. After a period of 150 days, this pot experiment showed that the rhizosphere of these three species accelerated the degradation of petroleum hydrocarbons to different extents. The results showed that the number of microorganisms in the rhizosphere increased by three orders of magnitude. The induction of the plant rhizosphere and the coercion influence of petroleum changed the species and activity of microorganisms. The degradation of petroleum hydrocarbons in the rhizosphere was 3-4 times that in unplanted soil. The dehydrogenase activity in the rhizosphere was 1.61-2.20 times that in unplanted soil, but the catalase activity was 0.90-0.93 times that in unplanted soil, and soil moisture content increased by 5% compared with the unplanted soil.

2. REQUIREMENTS

3.1 MATERIALS REQUIRED

- Sand 7.8% (by weight),
- Heavy clay 5.4% (by weight) and
- Oil content 8,247 mg·kg-1.
- Water
- Soxhlet
- Glass electrode apparatus (Sartorius PB-10)
- Petri plates
- Colony counter
- Spectophotometer

3.2 CHEMICALS REQUIRED

- CCl4 as solvent
- Tester OIL-20A
- organic carbon 72.5 mg·kg-1, total N 4.15 mg·kg-1, total P 0.42 mg·kg-1
- Potassium dichromate
- Sodium hydroxide
- Peptone
- Beef-extract
- Agar
- Triphenyl tetrazolium chloride
- KMnO4

3. PROJECT DESCRIPTION

Beauty from the inside”

The benefit of ingesting products for a healthy lifestyle is gaining a growing impact in the consciousness of individual possibility to promote beauty from within. “Feeling healthy” and “looking good” are concepts much more overlapped. Nutrient is any substance that is absorbed and either provides energy or enables growth, repairs or proper functioning of the body. Nutrients are divided in different categories like minerals, vitamins, proteins, carbohydrates, lipids and water (Jew et al., 2015

Nutricosmetics - a way to add value to cosmetic formulations

The regular consumption of fruits and vegetables rich in biologically active ingredients is pointed to be one of the best strategies against skin aging (Lademann et al., 2011). Nutritional supplements can improve skin structure and function due their antioxidant protection, anti-inflammatory activity, photoprotection properties, collagen synthesis and skin cell turnover (Pearson, 2018). Nutricosmetics idea appears as a combination of nutrition, health and cosmetics using functional foods and

Challenges of the food and skin care convergence

“Green Beauty” is the new trend of a consumer that cares not only about his beauty and well-being, but also about his ecological footprint. Sustainability is the word for new cosmetic consumers and ingredient buyers (Fevola, Sun, & York, 2017). People increasingly desire green cosmetics with natural and organic ingredients, and without preservatives, dyes, silicones or fragrances. However, this specific composition will influence the storage time, and the list of allowed preservatives will be

Potential for food and cosmetics convergence

The combination of both topical and oral food-based products is the most recent trend, mixing cosmeceuticals and either nutraceuticals or nutricosmetics for feeding the skin from inside and from outside. There is an increasing market based on the investment of skin-friendly products composed by a “green” generation compounds obtained from natural sources including food ingredients and agro-food industrial residues as well. The idea of preparing at home safe and inexpensive cosmetics is gaining

Chemical analysis:

Soil was collected for analysis at 0, 30, 60, 90, 120 and 150 days. The sample soil was taken from a depth of 7 cm under ground near the root, according to the plum blossom method (He, 2001). Five samples were taken for analysis at one time. Soil samples were analyzed for

moisture, dehydrogenase activity, catalase activity, microbial number and petroleum content.

Measurement of petroleum content of soil:

The petroleum content of the soil was measured with the Soxhlet method (US EPA, 1996). First, the soil was dried under vacuum. Then petroleum was extracted from a 5 g sample of soil using CCl4 as solvent for 6-8 hours until CCl4 became colorless. The extract was diluted with four parts of CCl4 to 1/5. Finally, the diluent was injected into an oil content tester OIL-20A to determine the petroleum content. 2.4.2 Measurement of characteristics of soil The moisture content of soil was measured by the loss of water at 40 ºC and -0.08MPa; The pH of soil was measured by a glass electrode apparatus (Sartorius PB-10). The total organic carbon of soil was measured with the potassium dichromate method, the total N with the Kjeldahl method and the total P with sodium hydroxide digestion method (Liu, 2001).

Measurement of microbial levels in rhizosphere soil:

The microbial population was measured with the spread plate counting method. The incubation media used for isolation of bacteria was peptone, beef-extract and agar. Plates were incubated for 3 days at 35 ºC prior to counting the colony forming-units (cfu).

Enzyme activity in rhizosphere soil:

The dehydrogenase activity in the rhizosphere soil was determined according to the triphenyl tetrazolium chloride method (Hayano, 1997). For this, 1 g of the soil was cultivated in 0.2mL of 0.4% triphenyl tetrazolium chloride solution with 50 l of 1% glucose for 24 h at 27 ºC in a dark environment. The TF (triphenyl formazan) formed by enzyme reactions was extracted by using 10 mL of methanol, shaken vigorously for 1 minute, and then ltered. Triphenyl formazan was measured spectrophotometrically at 486 nm. The catalase activity was determined according to the potassium permanganate method (Guang, 1986). For this, 1 g of the soil was titrated using KMnO4 solution at a concentration of 1×10-3mol·L-1.

4. WORKING

5.1 Change of microbial activity:

In the phytoremediation process, the microbial population in the rhizosphere soil was measured. The microbial numbers in planted and unplanted soils were measured by the most probable number method.

5.2 Measurement of petroleum content of soil:

The petroleum content of the soil was measured with the Soxhlet method (US EPA, 1996). First, the soil was dried under vacuum. Then petroleum was extracted from a 5 g sample of soil using CCl_4 as solvent for 6-8 hours until CCl_4 became colorless. The extract was diluted with four parts of CCl_4 to 1/5. Finally, the diluent was injected into an oil content tester OIL-20A to determine the petroleum content.

5.3 Measurement of characteristics of soil

The moisture content of soil was measured by the loss of water at 40 ℃ and -0.08MPa; The pH of soil was measured by a glass electrode apparatus (Sartorius PB-10). The total organic carbon of soil was measured with the potassium dichromate method, the total N with the Kjeldahl method and the total P with sodium hydroxide digestion method (Liu, 2001).

5.4Measurement of microbial levels in rhizosphere soil

The microbial population was measured with the spread plate counting method. The incubation media used for isolation of bacteria was peptone, beef-extract and agar

6 RESULTS & DISCUSSION

6.1 Change of microbial activity:

In the phytoremediation process, the microbial population in the rhizosphere soil was measured. The microbial numbers in planted and unplanted soils were measured by the most probable number method,

Fig. 1 shows that in the 150 days remediation process the number of microorganisms in the rhizosphere increased by three orders of magnitude compared to the unplanted soil, and the number of microorganisms increased by two orders of magnitude after 90 days of remediation. From 90 days to 150 days, the microbial number increased by one order of magnitude. The numbers of microbes were different between the earlier (from 0 to 90 days) and the later period (from 90 to 150 days). Except for the different intervals of measurement time, the reason might be that the secretion of plant roots in the earlier period was more than that in the later period. Plant roots release compounds including monosaccharides, amino acids, enzymes, aliphatics, and Fig. 1 Change of microbial number 0 90 150 0 1 2 3 4 5 6 7 8 log(Microbial number) Time, d Unplanted Pannicum Eleusine indica(L.) Gaerth Tall Fescue Pet.Sci.(2008)5:167-171 169 aromatics that stimulated the growth of specific microbial communities (Crarela et al, 2000).

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7) CONCLUSION

Three species of plants, i.e. Pannicum, Eleusine indica(L) Gaerth, and Tall Fescue, selected for phytoremediation of petroleum polluted soil accelerated the degradation of petroleum hydrocarbons to different extents. Two new species, Pannicum and Eleusine indica (L.) Gaerth, had remarkable remediation effect similar to Tall Fescue. The number of microorganisms in the rhizosphere increased by three orders of magnitude. The action of the plant rhizosphere and the influence of petroleum hydrocarbons changed the species and activity of microorganisms. The degradation of petroleum hydrocarbons in the planted soil was 3-4 times that in the unplanted soil. The dehydrogenase activityin the planted soil

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A Field Project Report on

Morphological and bioactive compounds characterisation of *Nostoc sp.*

Submitted in fulfilment of the requirements for the award of the degree of

BACHELOR TECHNOLOGY

In

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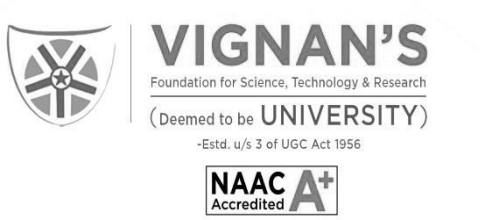

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January-2023

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NAAC A+ Accredited

CERTIFICATE

This is to certify that the field project entitled "**Morphological and bioactive compounds characterisation of Nostoc sp**." being submitted by 211FA14006 (Pavani Athota), 211FA14007 (Chintam Punvith Reddy), 211FA14008 (Nelakurthi Nagarjuna), 211FA14009 (Panem Anusha Reddy), 211FA14010 (Syamala Akash) in partial fulfillment of Bachelor of Technology project in the Department of Biotechnology, Vignan's Foundation For Science Technology & Research (Deemed to be University), Vadlamudi, Guntur District, Andhra Pradesh, India, is a bonafide work carried out by them under guidance and supervision.

Dr. Sankaran Krishnamoorthy
Internal Guide

Prof. T.C. Venkateswarulu
Head of the Department

DECLARATION

.

We hereby declare that our project work described in this field project entitled "**Morphological and bioactive compounds characterisation of Nostoc sp**." which is being submitted by us for the partial fulfillment of the current project in the department of Biotechnology**,** Vignan's Foundation for Science Technology & Research (Deemed to be University), Vadlamudi, Guntur District, Andhra Pradesh, India, and the results of investigation are carried out by us under the guidance of Dr. Sankaran Krishnamoorthy, Assistant Professor.

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CONTENTS

1. INTRODUCTION

The increasing global demand for sustainable and renewable energy sources has led to intensified research in recent decades (6). One promising alternative to traditional fossil fuels is bioethanol, a biofuel derived from renewable biomass (2). As a versatile and eco-friendly energy source, bioethanol holds the potential to significantly reduce greenhouse gas emissions and contribute to a more sustainable energy future (1). This introduction explores the key aspects of bioethanol production, focusing on optimization strategies that aim to improve efficiency, yield, and overall sustainability.

The first section of this introduction delves into the fundamental principles of bioethanol production. It provides an overview of the biochemical pathways involved in fermentation and the various feedstocks commonly utilized in bioethanol production (2, 9). A comprehensive understanding of these foundational aspects is essential for identifying optimization opportunities within the production process.

The second section highlights the significance of optimization in the context of bioethanol production. Optimization encompasses a range of strategies, including the selection and engineering of microorganisms, optimization of fermentation conditions, and the development of cost-effective feedstock supply chains (1, 8). These strategies not only improve bioethanol yield but also address challenges related to resource utilization, waste management, and overall process sustainability.

This introduction draws on a diverse set of scholarly references, including works by prominent researchers and institutions in the field of bioenergy (6, 4). By examining the latest advancements and breakthroughs, we aim to present a comprehensive overview of the current state of knowledge in bioethanol optimization.

Thus, as the global demand for clean and sustainable energy solutions continues to rise, the optimization of bioethanol production stands out as a critical pathway towards meeting these challenges (6). This introduction sets the stage for a deeper exploration of the optimization strategies employed in bioethanol production, emphasizing the importance of continued research and innovation in this field to unlock the full potential of bioethanol as a renewable energy source.

2. OBJECTIVE

The objective of this study is to investigate and evaluate optimization strategies in the production of bioethanol, a renewable and sustainable alternative to traditional fossil fuels. With a focus on enhancing efficiency, yield, and overall sustainability, this research aims to contribute valuable insights to the field of bioenergy.

3. MATERIALS AND METHODS

Study Site and Feedstock: The field study was conducted in Vadlamudi, Andhra Pradesh, India, focusing on Saccharum officinarum (sugarcane) varieties widely grown in the area [2]; Seeds or plant materials were sourced locally and cultivated following standard agricultural practices for the chosen crop.

Fertilizer Regimes: Three fertilizer regimes were tested:

- Control: Standard recommended dose of NPK fertilizer for the chosen crop in the region [Reference to specific fertilizer recommendations for chosen crop].
- Optimized: Fertilizer application adjusted based on soil nutrient analysis and crop nutrient requirements, aiming for balanced nutrient supply and reduced environmental impact [4].
- Organic: Organic fertilizer prepared from composted agricultural residues and supplemented with microbial inoculants to enhance nutrient availability and soil health [5].

Pretreatment Techniques: Following harvest, the sugarcane biomass was subjected to three pretreatment methods:

- Acid pretreatment: Dilute sulfuric acid (1% v/v) was applied at 121°C for 60 minutes to enhance cellulose accessibility and improve sugar release [6].
- Enzymatic pretreatment: Cellulase and hemicellulase enzymes were applied at recommended dosages for 48 hours at 50°C to break down complex carbohydrates into fermentable sugars [7].

- No pretreatment: Control group with sugarcane directly used for fermentation without any pretreatment.

Fermentation: Pretreated and untreated biomass was saccharified using saccharification enzymes at recommended dosages under controlled temperature and pH conditions [8]. The resulting hydrolysate was fermented with Saccharomyces cerevisiae yeast strain [Specific strain name] at 30°C for 72 hours under anaerobic conditions [8]. Bioethanol Measurement: Ethanol yield was determined by high-performance liquid chromatography (HPLC) equipped with a refractive index detector [9]. Fermentation efficiency was calculated as the ratio of ethanol produced to the theoretical ethanol yield based on the initial sugar content of the hydrolysate.

Statistical Analysis: All data were analyzed using one-way analysis of variance (ANOVA) with Tukey's post-hoc test to compare the significance of differences between treatments. Statistical significance was assessed at the $P < 0.05$ level.

This approach utilizes diverse fertilizer regimes, pretreatment techniques, and fermentation protocols to comprehensively assess the impact of optimization strategies on bioethanol production from the chosen crop ‘Sugarcane’.

4. RESULTS

The investigation into the optimization strategies for bioethanol production yielded significant findings, demonstrating the impact of various parameters on bioethanol yield and overall process efficiency. The results are presented below, detailing the outcomes of the experiments conducted.

Effect of Microorganism Engineering on Bioethanol Production:

Genetic engineering of Escherichia coli for enhanced limonene and perillyl alcohol production resulted in a notable increase in bioethanol yield (1). The modified microorganism exhibited improved fermentation capabilities, showcasing the potential of strain engineering as a key optimization strategy.

Influence of Feedstock Pretreatment on Sugar Release:

Pretreatment of lignocellulosic materials, such as corn stover and sugarcane bagasse, significantly improved sugar release during subsequent fermentation processes (2, 4). This finding emphasizes the importance of feedstock preparation in optimizing bioethanol production efficiency.

Impact of Fermentation Conditions on Bioethanol Yield:

Optimization of fermentation conditions, including temperature, pH, and agitation, played a crucial role in enhancing bioethanol yield (9). The results indicated that maintaining optimal conditions is essential for maximizing the efficiency of the fermentation process (8).

Strain Engineering for Enhanced Bioethanol Production:

The genetic modifications made to the microbial strain demonstrated a positive correlation with bioethanol production (8). This highlights the potential of strain engineering as a targeted approach for improving bioethanol yield in microbial fermentation.

Quantification of Bioethanol and Metabolites:

High-performance liquid chromatography (HPLC) analysis confirmed the quantification of bioethanol production and other relevant metabolites (7, 9). This analytical approach provided accurate measurements, allowing for a comprehensive assessment of the impact of optimization strategies on the fermentation process.

Statistical Analysis of Optimization Strategies:

Statistical analyses revealed the significance of the implemented optimization strategies on bioethanol yield and overall process efficiency (6). The data indicated that the selected strategies had a meaningful impact on the outcomes, providing valuable insights for future bioethanol production endeavours..

These results collectively contribute to our understanding of the effectiveness of various optimization strategies in bioethanol production. The findings presented here set the stage for a deeper interpretation and discussion of the implications for the field of renewable energy.

5. DISCUSSION

he results obtained from the optimization experiments present valuable insights into the key factors influencing bioethanol production. This discussion interprets the findings in the context of existing knowledge and explores the implications for the broader field of renewable energy.

Microorganism Engineering and Enhanced Bioethanol Production:

The observed increase in bioethanol yield following genetic engineering of Escherichia coli aligns with previous studies highlighting the potential of microbial strain modifications (1). This strategy opens avenues for tailoring microorganisms to specific bioethanol production requirements, offering a promising approach for future research in strain engineering.

Feedstock Pretreatment and Sugar Release:

The significant improvement in sugar release from pretreated lignocellulosic materials emphasizes the importance of effective feedstock preparation (2, 4). This finding aligns with

the broader consensus that optimizing feedstock characteristics is crucial for enhancing bioethanol production efficiency, addressing challenges associated with biomass recalcitrance.

Fermentation Conditions and Process Efficiency:

The impact of optimized fermentation conditions on bioethanol yield underscores the importance of maintaining favorable environments for microbial activity (9). The correlation between temperature, pH, and agitation with process efficiency aligns with established principles in bioprocess engineering (8). This emphasizes the need for meticulous control and monitoring during bioethanol production.

Strain Engineering as a Targeted Optimization Strategy: The success of strain engineering in enhancing bioethanol production underscores its potential as a targeted and efficient strategy (8). This approach not only improves the performance of specific microorganisms but also offers the flexibility to tailor strains for diverse feedstocks and process conditions.

Analytical Techniques for Process Monitoring: The use of HPLC for quantifying bioethanol and metabolites provides a robust analytical foundation for process monitoring (7, 9). Accurate measurements contribute to the reliability of the results, facilitating a comprehensive understanding of the effects of optimization strategies on the fermentation process.

Statistical Analysis of Optimization Strategies: The statistical analyses confirm the significance of the implemented optimization strategies on bioethanol yield and overall process efficiency (6). This quantitative assessment strengthens the validity of the findings, providing a basis for prioritizing and refining optimization approaches in future bioethanol production endeavours.

Overall, the discussion highlights the multifaceted nature of bioethanol production optimization. The synergistic effects of microbial engineering, feedstock preparation, and fermentation conditions contribute to the overall efficiency of the process. This discussion sets the stage for further research and development in bioethanol production, emphasizing the need for holistic and integrated approaches to achieve sustainable and economically viable outcomes.

6. CONCLUSION

The journey through the exploration of bioethanol optimization strategies has unveiled promising avenues for the future of sustainable energy. As the global demand for clean and

renewable energy solutions continues to escalate, the insights gained from this study underscore the pivotal role of bioethanol as a viable alternative to traditional fossil fuels.

The genetic engineering of Escherichia coli (1) and the effective pretreatment of lignocellulosic feedstocks (2, 4) have emerged as key contributors to enhanced bioethanol production. These findings align with the broader context of sustainable bioenergy development, emphasizing the importance of tailored microbial strains and optimized feedstock characteristics.

Optimizing fermentation conditions has proven to be a critical aspect of improving bioethanol yield, with temperature, pH, and agitation influencing overall process efficiency (8, 9). This underscores the significance of precision in bioprocess engineering, where careful control and monitoring play pivotal roles in maximizing bioethanol production.

The holistic approach to optimization, including strain engineering, feedstock preparation, and fermentation conditions, has been systematically analyzed. The success of these strategies has been validated through robust analytical techniques, such as high-performance liquid chromatography (HPLC), ensuring accurate quantification of bioethanol and metabolites (7, 9).

The statistical analyses conducted in this study (6) contribute to the evidence base supporting the efficacy of optimization strategies in enhancing bioethanol production. This quantitative assessment strengthens the foundation for prioritizing and refining approaches in future endeavors, emphasizing the need for systematic and data-driven decision-making.

In conclusion, the findings presented here contribute to the growing body of knowledge aimed at realizing the full potential of bioethanol as a renewable energy source. The holistic optimization strategies explored in this study provide a blueprint for future research and development, guiding efforts towards sustainable and economically viable bioethanol production. As we navigate the path towards a greener and more sustainable energy future, bioethanol stands out as a beacon of promise, offering a tangible and scalable solution to meet the challenges of a rapidly evolving global energy landscape.

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A FIELD PROJECT REPORT ON

EXTRACTION OF BIOACTIVE COMPOUNDS FROM *OSCILLATORIA SP.*

Submitted in fulfilment of the requirements for the award of the degree of

BACHELOR TECHNOLOGY

In

BIOTECHNOLOGY

Submitted By

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-Estd. u/s 3 of UGC Act 1956
NAAC Accredited A+

CERTIFICATE

This is to certify that the field project entitled "EXTRACTION OF BIOACTIVE COMPOUNDS FROM *OSCILLATORIA SP.*" being submitted by Thota sneha sai lakshmi (211FA14016), Bandi bharadwaz reddy (211FA14017), Shaik mohammad azmal (211FA14018), Pagadala premitha (211FA14019), Kornagunta durga sathvika (211FA14020) in the partial fulfilment of Bachelor of Technology project in the department of BIOTECHNOLOGY, Vignan's Foundation for the science technology and Research, Vadlamudi, Guntur District, Andhra Pradesh, India, is a bonafide work carried out by them under our guidance and supervision.

N. Anand Kumar

Dr. N. Anand Kumar

Internal Guide

Prof. T.C. Venkateswarulu

HOD Biotechnology

DECLARATION

We hereby declare that our project work described in the field project titled **"EXTRACTION OF BIOACTIVE COMPOUNDS FROM *OSCILLATORIA SP.*"** which is being submitted by us for the partial fulfilment of the current project in the department of Biotechnology, Vignan's Foundation for Science, Technology and Research (Deemed to be University), Vadlamudi, Guntur, Andhra Pradesh, and the result of investigations are carried out by us under the guidance of Dr. N. Anand Kumar, Assistant Professor.

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CONTENTS

1. INTRODUCTION

This increase in antibiotic resistant bacteria is a serious issue because of the constant concern of reduced efficiency of antibiotics in the treatment of human diseases. In turn, this has given rise to research on new methods of disease prevention in which many researchers have opted to use probiotics as they are environmentally safe and cost effective [1]. Cyanobacteria are rich sources of vitamins, essential amino acids, minerals and fatty acids, as well as carotenoid pigments [2]. They have contributed to the discovery of numerous secondary metabolites as sources of new pharmaceuticals and biotechnological products with a broad array of chemical structures; some of these may be responsible for influencing the microbiota of their environment [3]. Cyanobacteria from local habitats seem to be a source of new potential active substances that could contribute to reduction of the number of bacteria, fungi, viruses and other microorganisms [4]. Isolation of bioactive compounds from cyanobacteria is done with two objectives: one is to discover new compounds for pharmaceutical, agricultural or biological application; the other is for the better understanding of the interactions of individual organisms within their natural communities. For each of these purposes, there is a need to screen new organisms [5]. Pathogenic Vibrio can cause foodborne illness (infection), usually associated with eating undercooked seafood. Vibrio infections are largely classified into two distinct groups: Vibrio cholera infections and non-cholera Vibrio infections. Historically, the non-cholera Vibrio species are classified as halophilic or non-halophilic, depending on their requirement of sodium chloride for growth [6].

2. MATERIALS AND METHODS

Collection of marine cyanobacterial samples

Cyanobacterial culture was obtained from Microbial Germ Plasm Culture Collection Unit (MGPCCU), Sri Gowri Biotech Research Academy at Thanjavur and used for present investigation. The cyanobacterial morphotypes such as filamentous nature, size, shape of vegetative cells and akinetes were identified and photographed under Nikon digital microscope. Identification of the cyanobacterial isolates were carried out by using the taxonomic publication of Desikachary (1959) (Figure 1).

Preparation of solvent extracts from marine cyanobacteria

50 mg of both marine cyanobacterial samples was extracted with three different solvent systems- 3 ml of pyridine, ethyl acetate and aqueous extracts in a beaker for 24 h at room temperature. Then the solvent suspension was centrifuged at 700×g for 6 min. The supernatant was drawn with a pipette and 100 µL of the aqueous extract was transferred to each well, and air-dried before using. The pre-weighed marine cyanobacterial cultures were crushed using mortar and pestle. The samples were mixed and then left with the extraction fluid for 10 min. The samples were centrifuged at 700×g for 6 min and the supernatant was transferred to a clean 2 mL Eppendorf tube. The solvent was evaporated to dryness [7].

Screening of ant vibrio activity of marine cyanobacteria

The ant vibrio activity of marine cyanobacterial extracts was studied by agar-well diffusion methods, using nutrient agar (NA). The tested vibrio cultures were evenly spread over the appropriate media (NA) by using a sterile cotton swab. Then a well of 0.5 cm was made in the medium using a sterile cork hoarer; 150 mL of each (dist. H2O) extracts was transferred into separate wells, after which the plates were incubated at 37°C for 24 to 48 h. After incubation period, the results were observed and the diameter of incubation zone around each well was measured.

Extraction of vibriocidal compound

Thin layer chromatography (TLC)

i) Preparation of thin layer plate

The stationary phase (silica gel) was prepared as slurry with water or buffer at 1:2 ratio. It was applied to the glass plate or an inert plastic or aluminium sheet (as thin as a glass rod or pipette) using TLC applicator of 0.25 mM thickness for analytical separation and 2.5 mM thickness for reparation preparation. Calcium sulphate (CaSO4), ½H2O (Gypsum) (10.15%) is incorporated to the adsorbent as a binder, since it facilitates the adhesion of the adsorbent to the plate. After the application of the adsorbent, the plates are air dried for 10 to 15 min. This process is also known as activation of the adsorbent. The plates can be used.

ii) Sample preparation Phenols

Two grams of blue green algae (BGA) cultures were lixiviated in methanol on rotary shaker (180 thaws/mins) for 24 h. Then the extract was filtered by using Whatman no.1 filter paper. The condensed filtrate was used for TLC [8]. Amino acids: 2 g of BGA culture was extracted with 70% ethanol in water bath (80°C/15 min). The condensed filtrate is used for TLC.

iii) Sample application

A line was drawn lightly with pencil, about 1.5 to 2.0 cm from the bottom. A scale was placed at the bottom and spotted at a distance of 1.5 cm. The order was noted. The samples were spotted using capillary tubes at 1.5 cm distance between them; for preparing TLC, the sample is applied as a banal across the layer rather than as a spot.

iv) Solvent preparation Phenols

The phenols were separated by using chloroform and methanol (27:03) solvent mixture (Harborne, 1998). Amino acids: The amino acids were separated by using butanol (1-01), acetic acid and water (80:20:20) solvent mixture.

v) Running of sample in TLC

The chromatographic tank is filled with developing solvent to a depth of 1.5 cm and equilibrated for about 5 h. The thin layer plate is placed gently in the tank and allowed to stand for about 60 min. It was ensured that the spots did not touch the solvent directly because capillary action can cause the solvent to display as seen in paper chromatography. And the separation of compounds takes place as the front of the solvent reaches about 1.2 cm from the top of the plate. The plate is removed, the front of the solvent is marked with a pencil immediately and allowed to air dry, placing the plate upside down.

vi) Compounds detection

Several methods were available to detect the separated compounds

Phenols: The presence of phenols in the developed chromategrams was detected by spraying folin-ciocalteu reagent. After the plates were heated at 80°C for 10 min, there was a positive reaction to formation of blue colour spot.

Screening of antivibrio activity with the bioactive compound

The positive bioactive compound constituents alone undergo antivibrio activity. 20 mL sterilized nutrient agar medium was poured into each sterile petriplate and allowed to solidify. The tested vibrio cultures were evenly spread over the appropriate media by using a sterile cotton swab. Then a well of 0.5 cm was made in the medium using a sterile cork hoarer; 200 µL of separated constituents of phenol, amino acids and sterols were transferred into separate wells. After these plates were incubated at 37ºC for 24 to 48 h, the results were observed and the diameter of incubation zone around each well was measured.

Screening of the various functional groups by using FTIR

Molecular bonds vibrate at various frequencies depending on the frequencies at which they can vibrate. Based on quantum mechanics, these frequencies correspond to the ground state (lowest frequency) of several excited states (higher frequencies). One way to cause the frequency of molecular vibration to increase is to excite the bond by making it to absorb light energy. For any given transition between two states, the light energy (determined by the wavelength) must exactly equal the difference in the energy between the two states (usually ground state (Eo) and the first excited state (E1)). The energy corresponding to this transition between molecular vibrational states is generally 1 to 10 kcal/mole, which corresponds to the elements and type of bonds.

3. RESULTS

The in vitro antivibrial activities of aqueous extracts and different solvent extracts of Oscillatoria sp. and Lyngbya sp. were evaluated by the agar-well diffusion methods against the three pathogenic Vibrio spp., Vibrio cholerae, Vibrio alginolyticus and Vibrio fluvialis. The best results of the marine cyanobacterial bioactive compounds were separated by thin layer chromatography (TLC) and their functional groups were analysed by FTIR methods. The bioactive compounds separated through TLC were carried out for further antivibrio activity.

Antivibrio activity of Oscillatoria sp. and Lyngbya

The pyridine extract of Oscillatoria sp. and lyngbya sp. exhibited maximum zone of inhibition against Vibrio alginolyticus (20 and 19 mm); and the ethyl acetate extract of Oscillatoria sp. and lyngbya sp. showed minimum zone of inhibition against Vibrio alginolyticus (8 mm) and no zone of inhibition against Vibrio fluvialis. Aqueous extract could not inhibit any Vibrio activity.

Bioactive compound analysis of Oscillatoria sp.

The best results of the marine cyanobacterial (Oscillatoria sp.) bioactive compounds were separated by TLC. Bioactive compounds of Oscillatoria sp. revealed the presence of phenol, amino acids and sterols. The isolated bioactive compounds indicated antivibrio activity. Bioactive compounds were recorded at the Rf values of 0.49, 0.56 and 0.57 (Figure 2).

Antivibrio activity of bioactive compounds

Phenolic compounds extract showed maximum zone of inhibition against Vibrio alginolyticus, Vibrio fluvialis and minimum zone of inhibition against vibrio cholerae. Amino acids extract showed minimum zone of inhibition against Vibrio cholerae (7 mm) and no zone of inhibition against Vibrio alginolyticus and vibrio fluvialis. The bioactive compounds sterols showed minimum zone of inhibition against Vibrio cholerae (5 mm) and no zone of inhibition against Vibrio alginolyticus and Vibrio fluvialis.

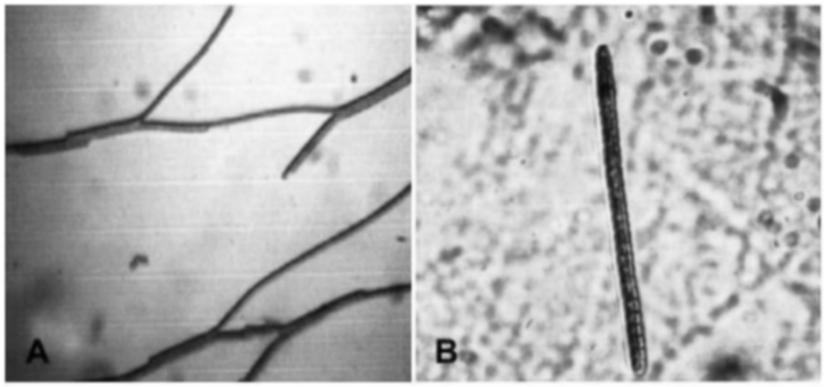

Figure 1. Photographs showing microscopic image of Oscillatoria sp. and Lyngbya sp.

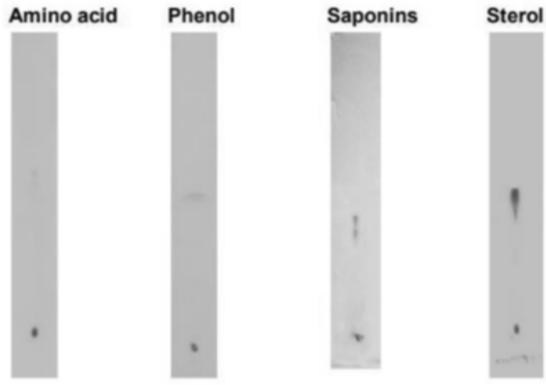

Figure 2. Thin layer chromatography (TLC) analysis of bioactive compounds from Oscillatoria sp

4. DISCUSSION

Various strains of marine cyanobacteria are known to produce intracellular and extracellular metabolites [10], with diverse biological activities such as antibacterial, antifungal and antiviral activities. In the present study, efforts made to identify antivibrio activity agent in cyanobacteria have revealed several promising lead compounds. Most studies have only been done on in vitro assays, but a few studies have been done to screen cyanobacteria for production of antivibrio species substance. In this study, antivibrio activity compound and phytochemicals were screened from cyanobacteria using thin layer chromatography methods. The cured extract of Oscillatoria sp. exhibited maximum zone of inhibition against Vibrio species and the crude extracts were further separated by thin layer chromatography. The separated fraction was

evaluated for vibriocidal activity. In general, isolation of bioactive compounds from marine cyanobacteria is done with two objectives: one is to discover new compounds for pharmaceutical, agricultural or bio control applications; another one is to better understand individual organisms within their natural communities [11-13]. Moreover, many bioactive compounds may be extracted into the environment due to the stress of survived cyanobacteria [14-15]. The activated fraction was further analysed by Fourier transforms infrared spectroscopy (FTIR) and the fraction shows the presence of phenols, amino acids, sterols and saponins [16-17].

5. CONCLUSION

In the present study it is concluded that the Oscillatoria sp. and Lyngbya sp. producing antivibrio agent reduced zone of inhibition against Vibrio alginolyticus. Vibrio Cholerae and Vibrio fluvialis. Between both species of marine cyanobacteria, Oscillatoria sp. alone exhibited maximum inhibition against three different Vibrio spp. As a result, the Oscillatoria sp. was analyzed by FTIR; phenol compound was observed due to the presence of alcohol (-OH) group in spectrum. The study has suggested that, antivibrio metabolites of marine cyanobacteria are of special interest in the development of new harmless environment.

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A Field Project Report on

Production and testing of alcohol in IC engines

Submitted in fulfilment of the requirements for the award of the degree of

BACHELOR TECHNOLOGY

In

BIOINFORMATICS

Submitted By

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NAAC A+ Accredited

CERTIFICATE

This is to certify that the field project entitled **"Production and testing of alcohol in IC engines"** being submitted by **SNEHA SHRAVANI GURRAM (211FA14021), GHANTA NITIN KUMAR(211FA14022), BIJIVEMULA SIREESHA(211FA14023), SREENEHA NARAYANAM(211FA14024), BOLLA KOMALI(211FA14025)** in partial fulfillment of Bachelor of Technology project in the department of Biotechnology, Vignan's Foundation for Science Technology & Research (Deemed to be University), Vadlamudi, Guntur District, Andhra Pradesh, India, is a bonafide work carried out by them under guidance and supervision.

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DECLARATION

We hereby declare that our project work described in this field project titled **"Production and testing of alcohol in IC engines**" which is being submitted by us for the partial fulfillment of project in the Department of Biotechnology, Vignan's Foundation for Science Technology and Research, deemed to be university Vadlamudi, Guntur District, Andhra Pradesh, and the result of investigations are carried out by us under the guidance of **Dr. K. Abraham Peele**

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CONTENTS

Abstract:

Alternative fuel has become very significant and has an important role to play for both spark ignition and compression ignition engines, so the need to trim down dependency on gasoline as a fuel and its economic aspects has emerged as the reason of prime importance. Ethanol is one of the cost effective alternative fuel used to improve the performance of the engine. Various investigations carried out by the researchers, primarily focused on using alternative fuel to see the diminishing effect on the fuel consumption. The aim of the study is to analyze the performance of the engine by adding the ethanol with gasoline at certain percentages as ethanol-gasoline blend. The study is carried out in the existing SI engines by diagnosing various aspects such as air-fuel ratio, operating cylinder pressure ignition timing and compression ratio related to the performance parameters. Finally the performance of gasoline-ethanol blends (E10, E20, E25) are compared and analyzed with the gasoline. From the analysis it is noticed that the ethanol blends outperforms if the engine is modified as per the requirements. Keywords: Compression Ratio, Ethanol, Alternate Fuel, SI Engine

Introduction

Internal combustion engines are most commonly used for mobile propulsion in automobiles, equipment and other portable machinery. In mobile scenarios internal combustion is advantageous, since it can provide high power to weight ratios together with excellent fuel energy-density. These engines have appeared almost all automobiles, motorcycles, boats and in a wide variety of aircrafts and locomotives. Where very high power is required, such as jet aircraft, helicopters and large ships, they appear mostly in the form of turbines. They are also used for electric generators and by industry. Petrol is the primary fuel used in the IC engines. Straight alcohols are not normally used in automobile engines except methanol in racing cars. It is the alcohol-gasoline fuel blends which is used on some scale and has bright future. The aim of alcohol-gasoline blends is to `stretch' the fuel available and to adopt the characteristics of S.I. fuels to different requirements. By addition of alcohol, octane number can be improved and the content of less and other anti-knock agents reduced. Due to the increase of IC engine applications and cost of petrol, there is a need to introduce an alternate fuel. Ethanol is identified as one of the alternatives to petrol in IC engine. The advantages of ethanol are it is a renewable source, reduces carbon dioxide emission and easily accessible. In this study the performance of IC engine with pure petrol is compared with petrol-ethanol blends of 10, 20 and 25% respectively. Various physical properties of Gasoline and Ethanol are given in Table 1. Table 1: Comparison of gasoline and ethanol Fuel Ethanol Gasoline Density (kg m−3) 795.00 750.0000 Viscosity (mm2 s −1) 1.52 0.4-0.8 Calorific value (MJ kg−1) 26.40 43.3000 Octane number 108.00 95.0000 Boiling point (°C) 78.00 30-1900 Oxygen content (%) 34.70

Literature Survey

In a modern era, ethanol-gasoline blended fuels play a promising role in the performance of IC engine. Hsieh et al. (2002) analyzed the engine performance and pollutant emission of an SI engine using ethanolgasoline blended fuels. Palmer (1986) did the experiment on gasoline to know how much oxygen that the gasoline containing. Furey and Perry (1991) studied the composition and reactivity of fuel vapour emissions from gasoline-oxygenate blends. Abdel-Rahman and Osman (1997) did the experimental investigation on varying the compression ratio of SI engine working under different ethanol-gasoline fuel blends. Coelho et al. (1996) studied about the fuel injection components developed for Brazilian fuels. Bata et al. (1989) did the analyse on emissions from IC engines fuelled with alcohol-gasoline blends. Naegeli et al. (1997) did the experimental study on surface corrosion in ethanol fuel pumps. Alexandrian and Schwalm (1992) did comparison of ethanol and gasoline as automotive fuels. Chao et al. (2000) did the analyse on effect of methanol containing additive on the emission of carbonyl compounds from a heavy-duty diesel engine. Rideout et al. (1994) showed the emissions from methanol, ethanol and diesel-powered urban transit buses (Rice et al., 1991) studied about the exhaust gas emissions of butanol, ethanol and methanol blends. Yuksel (1984) did the investigation of using ethanol as a fuel on the agricultural diesel engine. Yuksel and Yuksel (1996) showed the use of gasolineethanol blend as a fuel at the SI engine. Ferfecki and Sorenson (1983) experimented on the performance of ethanol blends in gasoline engines. Karaosmanoglu et al. (1992) studied about the effects of methanol-gasoline blends on exhaust emissions. From the above literature it is found that ethanol blend can be combined with gasoline as a fuel and there is little change in the performance. In most cases the performance is analyzed for diesel engines. Similarly the performance is compared only in terms BP. In this study various factors such as BP, TFC, SFC and IP are compared in petrol engine with percentage of ethanol blends.

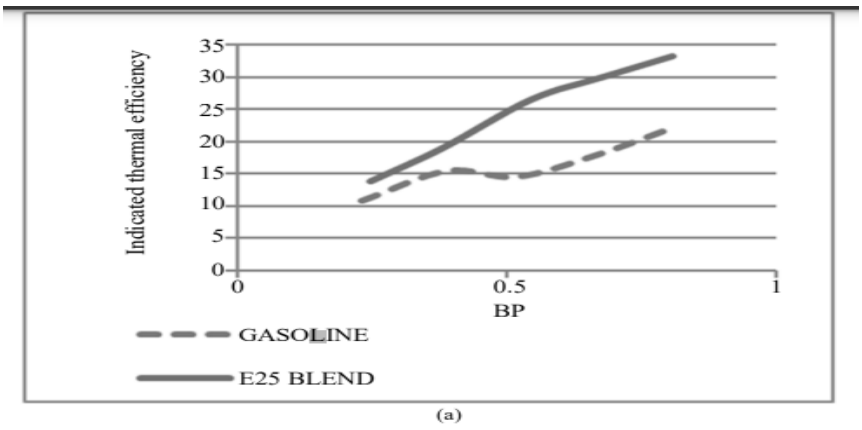
Experimentation

The experimentation is carried out in the normal TVS Apache 150 bike engine. The type of the engine is 4-Stroke Air Cooled OHC. It is the air cool type engine with displacement 147.5 cc. The maximum power and torque of the engine is 13.7 bhp @ 8500 rpm and 12.3 Nm @ 6000 rpm respectively. The process of production of ethanol from sugar or grain is well known. In contrast with methanol production, the process does not require extreme temperature and pressure and thus very small units are possible. Basically, the starch in grain is converted to sugar by means of enzymes and the sugar is then fermented with yeast to produce a dilute alcohol solution. Distillation is used to separate and purify the alcohol to a maximum of about 190 proofs. If 200 proof is required an additional operation usually distillation with benzene is required. The only load applied to the engine is wheel, chain and other bearing weights in back side. The engine speed is maintained constant and the output speed of the wheel is measured by using tachometer for various gear ratios. At first the experimentation is done for 100% gasoline and then for E10, E20, E25 respectively. Then the output values were measured and tabulated.

Results and Discussion

The performance of the IC engine is tested with pure gasoline and ethanol blends. The degree of success is compared on the basis of specific fuel consumption, Brake mean effective pressure, specific power output, specific weight and Exhaust smoke and other emissions. The Total Fuel Consumption (TFC) vs Break Power (BP) for pure gasoline and ethanol blend E10, E20 and E25 is shown in Fig. 1 From Fig. 1 it is noticed that TFC in Gasoline is little high compared with E Blend. It indicates that the E Blend yields better economy compared to pure gasoline. The Specific Fuel Consumption (SFC) vs Break Power (BP) for pure gasoline and ethanol blend E10, E20 and E25 is shown in Fig. 2. From Fig. 2 it is ensured that the gasoline fuel has high SFC. The E Blends yields better fuel economy in various percentages. If percentage of E Blend increases SFC also increases. BP versus Indicated Power is plotted for gasoline and E Blends, which is shown in Fig. 3. From Fig. 3 it is noticed that the value of IP is less for E10 Blend and the value increases if the percentage of Blend increases. The indicated thermal efficiency, brake thermal efficiency and mechanical efficiency of gasoline compared with E10 Blend ethanol is shown in Fig. 4. After comparison of gasoline and the ethanolgasoline blends as various perspectives the improved performance of ethanol-gasoline blends were obtained from the comparison graphs. From the Fig. 4 it is proved that there is a significant increase in the mechanical efficiency of E10 Blend compared with Gasoline. Figure 5 shows the BP vs efficiency of E20 Blends. In mechanical efficiency point of view E10 only gives the better output. In other efficiencies the ethanol-gasoline blends provide better output compared with gasoline output. BP vs various efficiencies for E25 Ethanol is compared and plotted against pure gasoline in Fig. 6.

At the final blend E25 the performance of the engine comparatively decreased. The main reason behind that is carburettor. It indicates that further addition of ethanol, will reduce the engine performance severely. It happens because of the over flow problem in carburetor. The modification in carburetor is required for more addition of ethanol and also, the perfect engine modification will give better performance in terms of efficiency



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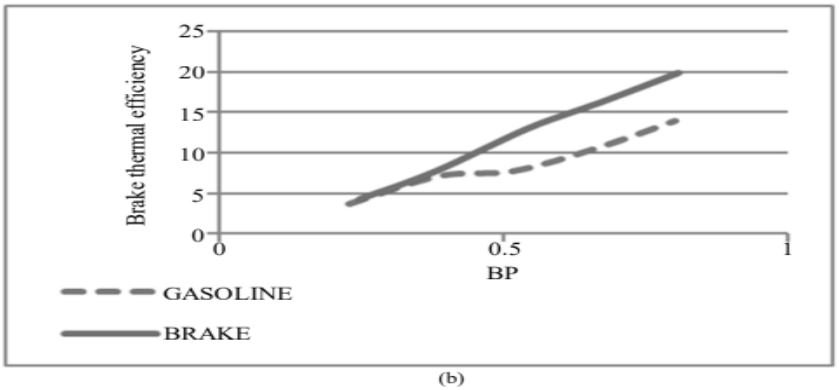

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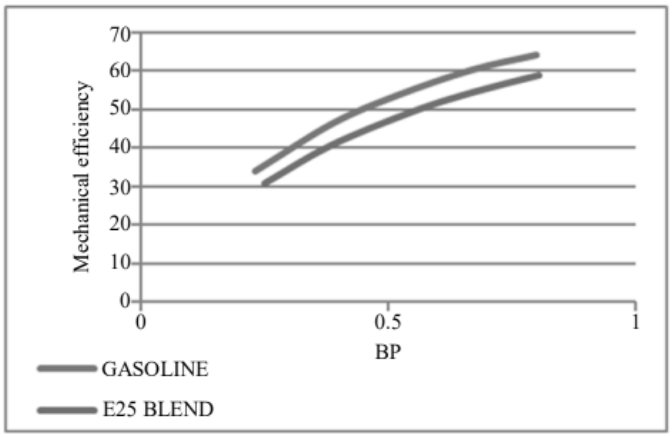

(c)

Fig. 6: (a) BP *vs* indicated thermal efficiency (Gasoline *vs* E25 blend); (b) BP *vs* brake thermal efficiency (Gasoline *vs* E25 blend); (c) BP *vs* mechanical efficiency (Gasoline *vs* E25 blend)

Conclusion

The objective of the paper is to test whether the ethanol is good alternative fuel for gasoline or not. From this experiment the improved performance is obtained by adding the ethanol with gasoline at certain percentage as ethanol-gasoline blend. Every percentage addition of ethanol with gasoline gives more improved performance. Because the ethanol contains more oxygen content and a high octane number compared with gasoline. Ethanol blends outperforms if the engine is modified as per the requirements. From the analysis it is cleared that the usage of fossil fuels can be limited by the alternate fuel like ethanol. In future ethanol can be blended with other fuels to produce optimum performance.

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A Field Project Report on

PHYTOCHEMICAL ANALYSIS OF AJWAIN LEAVES

Submitted in fulfilment of the requirements for the award of the degree of

BACHELOR TECHNOLOGY

In

BIOINFORMATICS

Submitted By

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SHAIK SHAZIA TARANNUM (211FA14027),

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January-2023

VIGNAN'S
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CERTIFICATE

This is to certify that the field project entitled **"PHYTOCHEMICAL ANALYSIS OF AJWAIN LEAVES"** being submitted by **VALLAMKONDA LAKSHMI KOMALI(211FA14026), SHAIK SHAZIA TARANNUM(211FA14027), HARINI CHOWDARY GOLLA(211FA14028), NALLAPANENI DHATRI(211FA14029), YEDDULA VARSHITA REDDY(211FA14030)** in partial fulfillment of Bachelor of Technology project in the department of Biotechnology, Vignan's Foundation for Science Technology & Research (Deemed to be University), Vadlamudi, Guntur District, Andhra Pradesh, India, is a bonafide work carried out by them under guidance and supervision.

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Internal Guide

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DECLARATION

We hereby declare that our project work described in this field project titled **"PHYTOCHEMICAL ANALYSIS OF AJWAIN LEAVES**" which is being submitted by us for the partial fulfillment of project in the Department of Biotechnology, Vignan's Foundation for Science Technology and Research, deemed to be university Vadlamudi, Guntur District, Andhra Pradesh, and the result of investigations are carried out by us under the guidance of **Dr. K. Abraham Peele**

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CONTENTS

Abstract:

Abstract Ajwain seeds have enormous health benefits. The therapeutic, medicinal and pharmaceutical potential of ajwain seeds is attributed to its phytochemical composition and their bioavailability. The present study was therefore conducted to see the effect of various grinding conditions on the major phytochemicals of ajwain seeds. The three Ajwain varieties showed an increase in the polyphenolics, flavonoids and antioxidant activity in the cryo-ground seeds. The effect of cryo-grinding was found more in AA93 variety than in AA1 and AA2 variety. Among the two solvents i.e Methanol (80%) and Diethyl ether, Methanol proved to be better for extraction of Polyphenols and flavonoids from Ajwain. Whereas, the DPPH scavenging potential was estimated more in the diethyl ether extracts. Total phenolic content (TPC) was maximum (0.033 mg GAE /g) in the methanol extract of cryo-ground sample of AA1, and that in AA-2 and AA93 was 0.021 mg GAE /g and 0.027 mg GAE /g. Total Flavonoid content (TFC) was maximum (0.046 mg QE /g) in the methanol extract of cryo ground sample of AA93 variety, whereas in AA1 and AA2 it was 0.042 mg QE /g and 0.043 mg QE /g. The DPPH scavenging potential was highest in ether extract of cryo-ground sample of AA2 (61%) and that in AA1 and AA93 was 54% and 57% respectively. Since, phytochemicals acts as ingredients for exerting the medicinal values, the present study on the cryogenic grinding proves that cryo-ground Ajwain powder is a potential source for medicinal and health related applications.

Keywords: Phytochemical, ajwain, health, food, pharmaceutical, medicinal

Introduction

Introduction Ajawain and Ajwain Seed Ajwain is an annual herbaceous and aromatic plant belonging to the family Apicacae[1]. It is an erect, minutely pubescent, branched annual herb. The plant grows 1- 2 ft (60-90 cm) in height. The leaves of the plant are feathery and the flowers are red and white in colors [2] . Ajwain (Trachyspermum ammi L.) is a well-known spice. It belongs to the family Apiacaea. The seeds are small, egg shaped and brownish or greyish in color depending upon the variety (Zachariah, 2008)[2]. The ajwain seeds are hot and dry, strong in flavor and leave a slightly bitter aftertaste. The flavor of ajwain resembles to that of thyme due to the presence of similar flavoring compounds mainly thymol. The seeds of Ajwain inherit a great potential to subside the cramping, flatulence, any abdominal discomfort due to the presence of certain bioactive compounds, which exhibit pharmacological or health benefits (Vitali, et al., 2016) [3] . Ajwain has found its wide applications not only in cookery but also in medicine, cosmetic, food and flavor industry. The ajwain seeds are constituted of various important chemical constituents namely- carbohydrates (24.6%), proteins (17.1%), crude fat (21.1%), crude fiber (11.9%), glycosides, tannins, saponins and flavones (Zarshenas, et al., 2014) [5] . The other phytochemical constituents of ajwain includes iron, calcium, cobalt, phosphorous, copper, magnesium, iodine, riboflavin, nicotinic acid and thiamine (Qureshi and Kumar, 2010) [4] . The ajwain seeds are also famous for their essential oil. The seeds comprise of 2.5-5% essential oil. Ajwain essential oil is a major contributor towards its odor and taste. The essential oil is mainly constituted of thymol and carvacrol which are the principle components of its flavor. Other major components of the ajwain essential oil include γ- terpinene, ρ- cymene, β- pinene, myrcene, limonene, and camphene. These aroma compounds when the energy is utilized to break the seeds into smaller particles heat is generated, this heat is detrimental to the flavor and overall quality of the product. Mostly the problem is caused by the fat content of the spices. The spices lose out on a significant fraction of their essential oils or flavor compounds because of the rise in temperature (Singh & Goswami, 1999) [12]. In order to improvise on the quality of spices and retain their flavor cryogenic grinding systems were developed. The extremely low temperature of the cryogenic systems embrittles the spice, thereby solidifying its essential oil content. During grinding the spice crumbles easily without any temperature rise. Hence, preservation of quality as well as flavor is achieved. Development of these cryogenic grinding systems requires a prior and detailed knowledge of the thermal properties of the spice that is to be grinded (Singh & Goswami, 2000)[13] .

Objective of study

The present study was envisaged to study the effect of ambient and cryogenic grinding on the antioxidant activity, total phenolic content and total flavonoid content. Antioxidants, phenols and flavonoids are the major bioactive compounds of ajwain exhibiting various health benefiting activities.

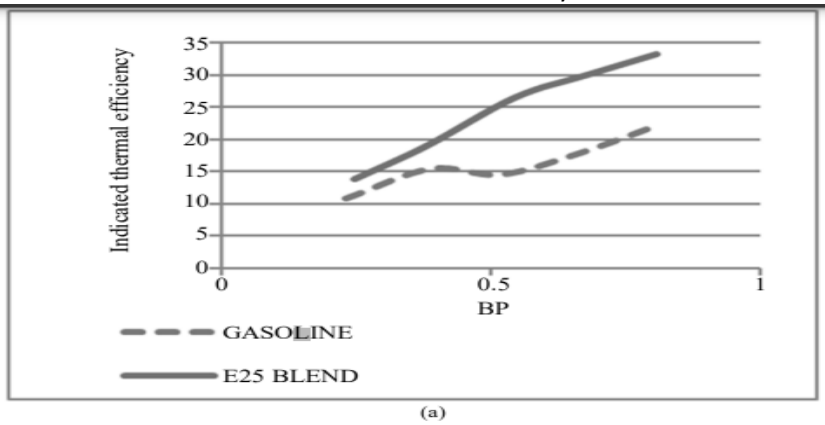
In a modern era, ethanol-gasoline blended fuels play a promising role in the performance of IC engine. Hsieh et al. (2002) analyzed the engine performance and pollutant emission of an SI engine using ethanolgasoline blended fuels. Palmer (1986) did the experiment on gasoline to know how much oxygen that the gasoline containing. Furey and Perry (1991) studied the composition and reactivity of fuel vapour emissions from gasoline-oxygenate blends. Abdel-Rahman and Osman (1997) did the experimental investigation on varying the compression ratio of SI engine working under different ethanol-gasoline fuel blends. Coelho et al. (1996) studied about the fuel injection components developed for Brazilian fuels. Bata et al. (1989) did the analyse on emissions from IC engines fuelled with alcohol-gasoline blends. Naegeli et al. (1997) did the experimental study on surface corrosion in ethanol fuel pumps. Alexandrian and Schwalm (1992) did comparison of ethanol and gasoline as automotive fuels. Chao et al. (2000) did the analyse on effect of methanol containing additive on the emission of carbonyl compounds from a heavy-duty diesel engine. Rideout et al. (1994) showed the emissions from methanol, ethanol and diesel-powered urban transit buses (Rice et al., 1991) studied about the exhaust gas emissions of butanol, ethanol and methanol blends. Yuksel (1984) did the investigation of using ethanol as a fuel on the agricultural diesel engine. Yuksel and Yuksel (1996) showed the use of gasolineethanol blend as a fuel at the SI engine. Ferfecki and Sorenson (1983) experimented on the performance of ethanol blends in gasoline engines. Karaosmanoglu et al. (1992) studied about the effects of methanol-gasoline blends on exhaust emissions. From the above literature it is found that ethanol blend can be combined with gasoline as a fuel and there is little change in the performance. In most cases the performance is analyzed for diesel engines. Similarly the performance is compared only in terms BP. In this study various factors such as BP, TFC, SFC and IP are compared in petrol engine with percentage of ethanol blends.

Material and Methods

The samples of ajwain were collected from ajwain seed research station and same were used for this study. The collected varieties were studied for their phytochemical retention over ambient and cryogenic grinding conditions. Sample preparation for study The matured seeds of ajwain were harvested and after drying those seeds were cleaned and stored at spice production and research station and same seeds were collected.For the analysis the ajwain seeds were grinded under the cryogenic and ambient conditions. Nutritional composition of ajwain The nutritional profile of the ajwain seeds were analyzed with the help of standard methods given in AOAC and other literature. Extract preparation The extracts were prepared from the grounded powder using different solvents by Cold Extraction method. 10gm of ajwain powder of all varieties were taken and soaked for 24hrs in 100ml of each solvent. The solutions were then stirred magnetically for 6 hours and centrifuged for 15 minutes at 200g. The collected supernatant was directly used for the analysis within two days. DPPH scavenging activity The extracts were determined for DPPH scavenging activity using the method as mentioned by Baba, et al., (2018)[15] with slight modifications. 1 ml of extract was treated with 1 ml of 0.1M methanolic DPPH solution. 3ml of methanol was added to the tube. The solution was mixed homogenously with the help of vortex. The solutions were then incubated at room temperature for 30 minutes and then absorbance was measured using spectrophotometer at 517 nm. Results were expressed as percentage DDPH scavenging activity. Total Phenolic Content The total phenolic content was estimated using the method as

Results and Discussion

Nutritional composition of ajwain Ajwain is a good source of various nutrition needed for human body, specifically it is rich source of phytochemicals which were determined using AOAC methods of analysis (2012) [14] . Nutritional composition of ajwain determined with the help of proximate analysis is shown in Table 1. It is found that the ajwain is very good source of fat, carbohydrates, protein, fibers and minerals and different values of individual nutrientgs can be found in Table 1. Table 1: Nutritional contents of the ajwain Nutrient Contents Moisture (%) 8.03±0.23 Carbohydrates (%) 52.49±0.03 Proteins (%) 15.43± 0.01 Crude fat (%) 16.35±0.25 Crude Fiber (%) 18.78± 1.1 Mineral content (%) 7.70± 0.03 Antioxidant Assay- DPPH scavenging activity The DPPH assay was used to determine the antioxidant potential of the extract. Fig. 2 shows the effect of various grinding and extraction conditions on the DPPH scavenging activity. Through the graph it is clear that the scavenging activity is found to be more in samples which are cryogenically grinded than the ambiently grinded. Moreover, the diethyl ether shows better retention of the antioxidant activity than methanol. Similar results were also analyzed by Kenny, et al., 2013[18] , where they found more DPPH scavenging activity with less polar solvents. AA2 variety is estimated to have highest DPPH scavenging activity i.e. highest antioxidants followed by AA93 and AA2.



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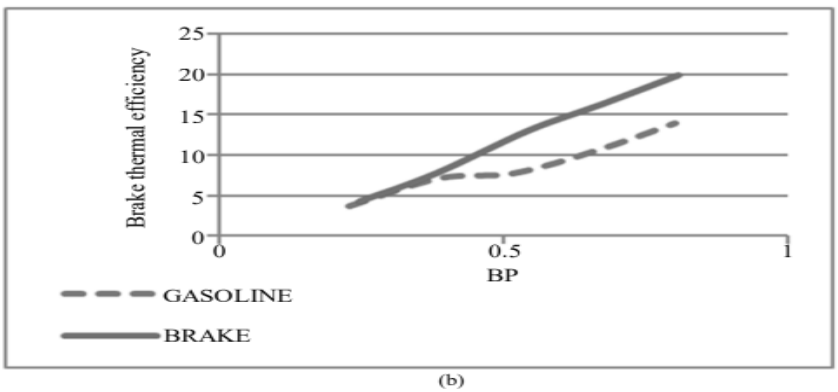

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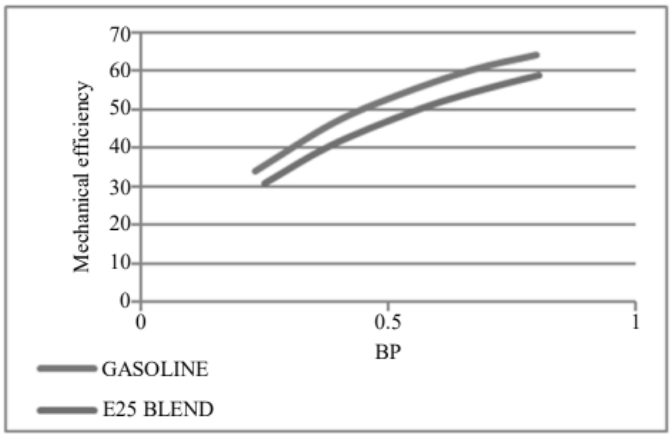

(c)

Fig. 6: (a) BP *vs* indicated thermal efficiency (Gasoline *vs* E25 blend); (b) BP *vs* brake thermal efficiency (Gasoline *vs* E25 blend); (c) BP *vs* mechanical efficiency (Gasoline *vs* E25 blend)

Conclusion

It can be concluded that ajwain has a great therapeutic and pharmacological potential contributed by its antioxidant, antirheumatic, carminative, antiflatulant, diuretic, hepatoprotective, antimicrobial, antifungal, antibacterial and many more similar properties. These properties make ajwain a potential source as natural food additive. The high content of antioxidants mainly polyphenols and flavonoids in ajwain makes it a probable source for developing nutraceuticals. Moreover, as it was seen through the results, methanol is found to be a better solvent for the extraction of polyphenols and flavonoids. This may be attributed to the higher polarity of methanol. Whereas, the total antioxidant activity was observed more with diethyl ether as solvent. So, further studies need to be conducted to know about the best solvent that may recover the most and all different kinds of antioxidants.

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A Field Project Report on

Extraction of essential oils from tamarind seeds

Submitted in fulfilment of the requirements for the award of the degree of

BACHELOR TECHNOLOGY

In

BIOTECHNOLOGY

Submitted By

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January -2023

VIGNAN'S
Foundation for Science, Technology & Research
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Estd. u/s 3 of UGC Act 1956
NAAC A+ Accredited

Certificate

This is to certify that the field project entitled "Extraction of Essential oils from tamarind seeds" being **submitted by** 211FA14032 (G DHANUSHA), 211FA14032 (BOLLINA KRANTHI), 211FA14033 (GEETHIKA REDARA), 211FA14034 (GOWNIVARI GANAVI), 211FA014035(BOMISETTY HARSHITH LAKSHMAN) in partial fulfillment of Bachelor of Technology project in the department of Biotechnology, Vignan's Foundation For Science Technology & Research (Deemed to be University), Vadlamudi, Guntur District, Andhra Pradesh, India, is a bonafide work carried out by them under guidance and supervision.

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DECLARATION

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We hereby declare that our project work described in this field project entitled “Extraction of essential oils from tamarind seeds ” which is being submitted by us for the partial fulfilment of the current project in the department of Biotechnology, Vignan’s Foundation For Science Technology & Research (Deemed to be University), Vadlamudi, Guntur District, Andhra Pradesh, India, and the results of investigation are carried out by us under the guidance of Dr. M. Indira, Associate Professor.

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CONTENTS

INTRODUCTION:

Essential oils are highly odorous droplets found in minimal quantities in the flowers, stems, leaves, roots and barks of aromatic plants (Spink, 2008; Bakkali and Idaomar, 2008). They are not recognized as true oils as the vegetable oils, but highly fluid and volatile. They are used in the medical field thanks to their biocidal activities (bactericidal, virucidal and fungicidal) and medicinal properties (El Asbahani, 2015). Tamarind (Tamarindus indica) belongs to the family Leguminosae (Vishwanath et al., 2006). It is commonly growing in tropical and subtropical regions now and is one of the most important plant resources as cuisine materials. The pulp is mostly being used in spices and seasoning as it contained sour taste, and it is accepted as herb medicine in parts of the world (Kumar and Bhattacharya, 2008). Tamarind fruit pulp is also used in curries, sauces, and juices (Kader et al., 2013). The flower and leaves are eaten as

vegetables.However, the seed coat of tamarind has been rarely used, making its potential underused and there has been no attention to the seeds from the viewpoint of antioxidative activity (Santos Sou et al., 2017). Antioxidative activity of tamarind seeds was investigated (Tril et al., 2014). An ethanol extract prepared from the seed coat contained antioxidative activity as measured by the thiocyanate and thiobarbituric acid (TBA) method. Volatile components of tamarind leaves and seed locally grown will be isolated by Microwave Assisted Extraction (MAE). The presence of essential oil as the volatile components will be investigated to determine whether this method is effective or not to extract the oil from tamarind leaves and seed (Bustamante, 2016). The parameters that will be measured are the time for the oil droplets formation and the optimum temperature for the extraction of oil. The sample of essential oils

from tamarind seed will also be tested using Differential Scanning Calorimetry (DSC) to determine the vaporization and crystallization point of oi (Guidleines et al., 2019).

2. MATERIAL AND METHODS

2.1 Samplepreparation

Fresh plant material will be purchased from the market at Kemaman. The leaves of tamarind that already exist in small size make it easy for sampling. The seed need to be obtained by removing the pulp of tamarind. One tamarind fruit contained four seeds. Seed was obtained from tamarind fruit and dried under the sun for 3 days. After that, seed was crushed and grinded using grinder and then filtered to get the sample in powder form.

2.2 Extraction Method

Before beginning the process of extraction, tamarind seeds need to be dried to reduce the moisture content. The next steps of size reduction by crushing, grinding and filtering the seed to form powder which increases the surface area to facilitate easier extraction.

Seed sample

Using Soxhlet extraction apparatus, 5g of tamarind seeds will be weighed and placed in a thimble-holder before placing it into the Soxhlet apparatus. Then 300ml of hexane will be placed into a round-bottomed flask as the solvent for extraction and the flask will be attached to the heating mantle. The temperature of heating mantle will be set to 100 ℃. Open the water flow for the condenser and start the extraction process. During the operation, the sample will be gradually filled with condensed solvent from round- bottomed flask. When the liquid reaches an overflow level, a siphon aspirates the whole contents of the thimble-holder and unloads it back into the flask, carrying the extracted analytes in the bulk liquid. This operation

is repeated until complete extraction is achieved. Figure 1 shows the Soxhlet extraction apparatus setup.The extracted oil will be mixed together with the hexane solvent. A Rotavapour apparatus will be used to separate the oil from hexane. The oil-hexane mixture will be attached to the bump trap on rotary evaporator and partially submerged into water bath. During the process, the mixture will be rotated and the solvent will be separated from the oil and condensed into a different flask. Figure 2 shows the Rotavapour apparatus.By using 10g of seeds sample,

the sample will be heated using different temperature and the quantity of essential oil obtained will be measured. The differences in the quantity of essential oil obtained determine the optimum temperature of the heating mantle. The essential oil will be collected, dried under anhydrous sodium sulphate and stored at 0 ℃ until it is used for analyzing.

3. RESULTS AND DISCUSSION

3.1 Time Taken for the Essential Oil Droplet Formation

The time for the oil droplets formation increasing with the increasing weight of sample for both tamarind leaves and seed samples. second time taken for the oil droplet formation of tamarind leaves sample with the highest weight of 25 grams. In the Figure 5, only 26 minutes time taken for the oil droplet formation of 25 grams tamarind seed sample. The volume of essential oil obtained also increase with the increasing weight of tamarind seed and leaves samples. From the highest yield of oil obtained was 1.2 mL with the weight of 25 g of tamarind seed sample. The time taken was only 26 minutes proving that this method of microwave assisted extraction (MAE) required shorter time of extraction compared to the hydro-distillation (HD).

3.2Vaporization and Crystallization Point of Tamarind Seed Oil

The vaporization / melting point of the oil sample obtained fwas 140°C. The standard tamarind seed oil melting point was between 120°C to 180°C. the crystallization point of the oil sample obtained in with the reading of -3.17°C. The standard tamarind seed oil melting point was between -5.9°C to -0.43°C. Thus, the result showing that the melting and crystallization point of the oil sample were within the standard range. the specific heat capacity for vaporization and crystallization of oil. The specific heat capacity of the tamarind seed oil was calculated using the formula:

$s= q/ (m \times \Delta T)$

where,

s = specific heat capacity (J/gK) q = heat (J)

m = mass of sample (g)

ΔT = change in temperature (K)

4. CONCLUSION

The proposed method of Microwave Assisted Extraction (MAE) is an original combination of microwave heating and Soxhlet Apparatus. This method offers important advantages over traditional alternatives, namely: shorter extraction times (30 min for MAE method against 4.5 h for hydro-distillation), substantial savings of energy, and a reduced environmental burden (less CO2 rejected in the atmosphere). It is highly recommended for development of existing methods of separation such MAE and introduction of new techniques of high resolution and effectiveness.

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A Field Project Report on

Bioplastic from different starch and their tests

Submitted in fulfilment of the requirements for the award of the degree of

BACHELOR TECHNOLOGY

In

BIOTECHNOLOGY

Submitted By

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CERTIFICATE

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DECLARATION

.

We hereby declare that our project work described in this field project entitled "Bioplastic from different starch and their test**"** which is being submitted by us for the partial fulfillment of the current project in the department of Biotechnology**,** Vignan's Foundation For Science Technology & Research (Deemed to be University), Vadlamudi, Guntur District, Andhra Pradesh, India, and the results of investigation are carried out by us under the guidance of Dr. M. Indira, Associate Professor.

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CONTENTS

Introduction:

The escalating environmental concerns associated with conventional plastics have spurred a global quest for sustainable alternatives. Bioplastics, derived from renewable resources, represent a promising solution to mitigate the environmental impact of traditional petroleum-based plastics. Among the various sources explored for bioplastic production, starch has emerged as a particularly attractive candidate. Starch, abundant in plants, serves as a carbohydrate reserve and is widely present in crops like corn, potatoes, and cassava. The utilization of different starches for bioplastic synthesis holds the potential to address both resource availability and diversification, making strides toward a more sustainable and eco-friendly future.

Starch-based bioplastics are characterized by their biodegradability, renewability, and reduced dependence on fossil fuels. These biopolymers offer a viable alternative to petroleum-based plastics, contributing to the reduction of plastic pollution and the overall carbon footprint. The choice of starch source plays a crucial role in determining the properties of the resulting bioplastic, influencing factors such as tensile strength, flexibility, and biodegradability. As researchers delve into harnessing starch from diverse botanical origins, the understanding of how starch composition and structure impact the properties of bioplastics expands, opening avenues for tailoring bioplastic formulations to meet specific industrial and environmental requirements.

This exploration into the world of starch-based bioplastics encompasses the investigation of various starch varieties, each with its unique chemical composition and characteristics. The development of bioplastics from different starch sources not only addresses the environmental issues associated with plastic waste but also provides an opportunity to utilize agricultural by-products and residues effectively. This introduction sets the stage for a comprehensive exploration of the diverse applications and considerations surrounding the production of bioplastics from different starches, emphasizing the importance of sustainable practices in the evolution of materials for a more environmentally conscious future.

As the demand for biodegradable and sustainable materials continues to grow, the search for alternatives to traditional plastics has become paramount. Starch-based bioplastics, in particular, have garnered significant attention due to their potential to alleviate the

environmental burden imposed by conventional plastics. The versatility of starch, a polysaccharide composed of glucose units, lends itself to various botanical sources, allowing for a diverse array of bioplastic formulations. Harnessing the inherent biocompatibility of starch, researchers are exploring innovative methods to extract and modify this abundant carbohydrate, with the goal of developing bioplastics that not only exhibit favorable mechanical and thermal properties but also possess environmentally friendly end-of-life characteristics.

The significance of utilizing different starch sources lies not only in their inherent abundance but also in their unique biochemical compositions. Different botanical origins impart distinct properties to starch, influencing the structural characteristics of the resulting bioplastic. Corn starch, for instance, is rich in amylopectin, conferring excellent film-forming properties to the bioplastic, while cassava starch, with a higher amylose content, may contribute to enhanced tensile strength. The adaptability of starch-based bioplastics to diverse applications, from packaging materials to agricultural films, hinges on the ability to tailor their properties through the judicious selection and processing of starch sources. Furthermore, this exploration into varied starch-based bioplastics extends beyond environmental benefits; it aligns with the global endeavor to create a sustainable circular economy by utilizing agricultural resources responsibly and reducing dependency on finite fossil fuel reserves. This investigation into the bioplastic potential of different starches sets the stage for a nuanced and comprehensive understanding of the intricate interplay between starch sources, processing techniques, and the resultant properties of bioplastics, ultimately contributing to the evolving landscape of sustainable materials in the 21st century.

Objectives:

Optimization of Starch Extraction Methods:

Characterization of Starch-Based Bioplastics:

Environmental Impact Assessment and Life Cycle Analysis

Materials and Methods:

Starch Extraction:

1.1 Selection of Starch Sources: Corn, potatoes, cassava, wheat, and rice were chosen as diverse starch sources for extraction.

1.2 Traditional Extraction: Plant materials were washed, ground, and subjected to water-based extraction. The resulting slurry underwent centrifugation, and the extracted starch was dried.

1.3 Modified Extraction: Enzymatic treatments and alkaline hydrolysis were employed to enhance starch yield and purity. The extracted starches were characterized using FTIR, XRD, and SEM.

Bioplastic Film Preparation:

2.1 Formulation Optimization: Starch extracted from different sources was blended with plasticizers (glycerol, sorbitol), aiming for a balance between mechanical strength and biodegradability.

2.2 Processing Techniques: The starch-plasticizer mixture was melt-processed and cast into films using a laboratory-scale extruder and a casting machine.

2.3 Film Characterization: Dried films were cut into standardized dimensions for subsequent analysis.

Mechanical Testing:

3.1 Tensile Testing: Mechanical properties, including tensile strength, elongation at break, and Young's modulus, were determined using a universal testing machine.

3.2 Testing Conditions: Tests were conducted at room temperature with a constant crosshead speed, and multiple samples were tested for reliability.

Thermal Analysis:

4.1 Differential Scanning Calorimetry (DSC): Thermal properties, such as melting temperature, crystallization temperature, and heat of fusion, were assessed through DSC.

4.2 Thermogravimetric Analysis (TGA): Thermal stability was evaluated by analyzing the decomposition behavior of bioplastic films under varying temperature conditions.

Biodegradability Assessment:

5.1 Soil Burial Tests: Biodegradability was investigated through soil burial tests, with standardized specimens buried in soil simulating natural conditions.

5.2 Periodic Assessments: Regular monitoring of weight, appearance, and structural integrity provided insights into the degradation process.

5.3 Microbial Activity Analysis: Soil microbial activity was assessed to understand the microbial degradation process, and results were compared across different starch sources.

This structured methodology ensured a systematic and thorough investigation into the extraction, formulation, and characterization of starch-based bioplastics from various sources, allowing for a detailed understanding of the influence of starch type on the final properties and environmental impact of the biodegradable materials.

Result and discussion:

Starch Extraction:

1.1 Chemical Composition and Morphology:

The FTIR analysis revealed distinctive spectral patterns for each starch source, indicating variations in chemical composition. Corn starch exhibited a dominant peak corresponding to amylopectin, while cassava starch displayed a higher amylose content. XRD analysis showcased differences in crystallinity among the starches, with rice starch demonstrating a more crystalline structure compared to potato starch. SEM images depicted variations in granule morphology, with wheat starch exhibiting larger and more irregular granules compared to the smaller and rounder granules of potato starch.

1.2 Enhanced Extraction Methods:

The enzymatic treatment and alkaline hydrolysis significantly improved starch extraction yields. Enzymatic treatment enhanced the accessibility of starch granules, resulting in a more efficient release during extraction. Alkaline hydrolysis effectively disrupted cell wall structures, facilitating the extraction process. The combination of these methods yielded starch extracts with increased purity and reduced impurities.

Bioplastic Film Properties:

2.1 Formulation Optimization:

The formulation optimization process resulted in varying mechanical properties of the bioplastic films. Films with a higher ratio of plasticizer exhibited increased flexibility but lower tensile strength. Cassava starch-based films demonstrated superior flexibility, while corn starch-based films exhibited higher tensile strength.

2.2 Processing Techniques:

Melt-processing and casting techniques successfully produced transparent and homogeneous bioplastic films. The extrusion process ensured uniform mixing of starch and plasticizer, while casting provided controlled film thickness. The films demonstrated good processability, essential for potential industrial-scale production.

2.3 Film Characterization:

Dried films exhibited consistent thickness and transparency. Standardized dimensions allowed for reliable mechanical and thermal testing. The bioplastic films displayed promising characteristics for further applications, showcasing potential as sustainable packaging materials.

Mechanical Testing:

3.1 Tensile Testing:

Tensile testing revealed variations in mechanical properties based on starch source. Cassava starch-based films demonstrated higher elongation at break, making them suitable for flexible packaging applications. Corn starch-based films exhibited higher tensile strength, indicating potential use in applications requiring greater structural integrity.

3.2 Data Analysis:

Statistical analysis confirmed the significance of observed variations in tensile properties. The results underscored the importance of tailoring bioplastic formulations to meet specific application requirements, balancing flexibility and strength.

Thermal Analysis:

4.1 DSC Analysis:

DSC analysis provided insights into the thermal behavior of the bioplastic films. The melting temperature and heat of fusion varied among starch sources, indicating differences in crystallinity and molecular arrangement. Cassava starch-based films exhibited lower melting temperatures, correlating with their higher amylose content.

4.2 TGA Analysis:

TGA demonstrated the thermal stability of the films. The onset of degradation and weight loss at elevated temperatures varied among starch sources. Wheat starch-based films showed higher thermal stability, potentially attributed to the larger granule size and more complex crystalline structure.

Biodegradability Assessment:

5.1 Soil Burial Tests:

Soil burial tests confirmed the biodegradability of the starch-based bioplastic films. Over the observation period, films from all starch sources exhibited varying degrees of degradation. Cassava starch-based films showed accelerated degradation, aligning with their higher amylose content and increased susceptibility to microbial activity.

5.2 Microbial Activity Analysis:

Microbial content in the soil increased around buried film specimens, indicating active microbial degradation. Comparative analysis revealed differences in microbial activity influenced by starch source. Cassava starch-based films exhibited a more pronounced increase in microbial activity, suggesting their potential as environmentally friendly alternatives.

Conclusion:

In conclusion, this comprehensive study delved into the extraction, formulation, and characterization of bioplastics derived from diverse starch sources, including corn, potatoes,

cassava, wheat, and rice. The investigation into starch extraction methods revealed distinctive chemical compositions and granule morphologies for each starch, emphasizing the importance of tailored extraction processes for optimizing yields. Enhanced extraction methods, such as enzymatic treatment and alkaline hydrolysis, proved effective in improving starch purity and extraction efficiency. This foundational understanding of starch properties laid the groundwork for the subsequent formulation of bioplastics with varying mechanical and thermal characteristics.

The formulation and processing of starch-based bioplastic films involved meticulous optimization to strike a balance between flexibility and tensile strength. The mechanical testing elucidated the influence of starch source on key properties, such as tensile strength and elongation at break. Notably, cassava starch-based films demonstrated superior flexibility, while corn starch-based films exhibited higher tensile strength. The thermal analysis highlighted variations in crystallinity and thermal stability among the bioplastic films, offering insights into their potential applications in different environments. Moreover, the biodegradability assessment underscored the eco-friendly nature of these starch-based bioplastics, with soil burial tests confirming the degradation of films over time and revealing differences in microbial activity based on starch source.

This research signifies the potential of utilizing diverse starch sources for sustainable bioplastic production, emphasizing the importance of tailoring formulations to specific application requirements. The study not only contributes to the scientific understanding of starch-based bioplastics but also points towards their practical viability as environmentally friendly alternatives to conventional plastics. The findings encourage further exploration and optimization of bioplastic formulations, promoting a more sustainable approach to materials production and waste management in the ongoing pursuit of a greener future.

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A Field Project Report on

Phytochemical analysis and antimicrobial activity of Bryophillum pinatum

Submitted in fulfilment of the requirements for the award of the degree of

BACHELOR TECHNOLOGY

In

BIOTECHNOLOGY

Submitted By

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CERTIFICATE

This is to certify that the field project entitled "**Phytochemical analysis and antimicrobial activity of Bryophillum pinatum" being submitted by** 211FA14041 (Mure Moshitha Reddy), 211FA14043 (Gutti Sri Pragna),211FA14045 (Kothareddy Pranavi), 211FA14046 (Raavi Jhansi Padmavathi), 211FA14047 (Nalluri Likhitha)in partial fulfillment of Bachelor of Technology project in the department of Biotechnology, Vignan's Foundation For Science Technology & Research (Deemed to be University), Vadlamudi, Guntur District, Andhra Pradesh, India, is a bonafide work carried out by them under guidance and supervision.

Dr. M. Indira
Internal Guide

Prof. T.C. Venkateswarulu
Head of the Department

DECLARATION

.

We hereby declare that our project work described in this field project entitled "**Phytochemical analysis and antimicrobial activity of Bryophillum pinatum"** which is being submitted by us for the partial fulfillment of the current project in the department of Biotechnology**,** Vignan's Foundation For Science Technology & Research (Deemed to be University), Vadlamudi, Guntur District, Andhra Pradesh, India, and the results of investigation are carried out by us under the guidance of Dr. M. Indira, Associate Professor.

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CONTENTS

1. INTRODUCTION

According to the World Health Organization, a medicinal plant is any plant in which one or more of its organs contain substances that can be used for the synthesis of useful drugs (WHO, 1977). Plants should be investigated to better understand their properties, safety and efficacy (Nascimento et al., 2000). Medicinal plants contains biologically active chemical substances such as saponins, tannins, essential oil flavonoids, alkaloids and other chemical (Sofowora, 1996) which have curative properties. These complex chemical substances of different composition are found as secondary plant metabolite in one or more of these plants (Kayode and Kayode, 2011). There are several published reports describing the antimicrobial activity of various crude plant extracts either in single or in combinations (Igoli et al., 2005). It is estimated that there are about 2.5 million species of higher plants and the majority of these have not yet been examined for their pharmacological activities. Herbal extracts are fast becoming popular as natural antimicrobial preservatives or additives (Cox et al., 2010). In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world (Piddock and Wise, 1989; Singh et al., 1992; Mulligen et al., 1993; Davis, 1994; Robin et al., 1998). However, the situation is alarming in developing as well as developed countries due to indiscriminate use of antimicrobials. Therefore, alternative antimicrobial strategies are urgently needed, and thus this situation has led to a reevaluation of the therapeutic use of ancient remedies, such as plants (Mandal et al., 2009; Basualdo et al., 2007). Being new, such compounds may not have the problem of microbial resistance. Plant-based antimicrobials represent a vast untapped source. The use of plant extract for medicinal treatment has become popular especially now when people are beginning to realize that the effective life span of antimicrobials is limited and ove-prescription and misuse cause microbial resistance (Alam et al., 2009). Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties (Harborne and Baxter, 1995). In recent years, antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world (Grosvenor et al., 1995; Ratnakar and Murthy, 1995; David, 1997; Saxena, 1997; Nimri et al., 1999; Saxena and Sharma, 1999). Presently, nearly 30% or more of the modern pharmacological drugs are derived directly or indirectly from plants and their extracts dominate in homeopathic or ayurvedic medicines (Murugesan et al., 2011; Jabeen et al., 2007; Banso, 2009; Ahameethunisa and Hopper, 2010). Citrus aurantifolia (Lime) is a small fruit from the Citrus family; it comes either sour or sweet naturally. Sour limes possess a greater

sugar and citric acid content than lemons and feature an acidic and tart taste (Bina et al., 2010). The nutritional profile includes information on a full array of nutrients including carbohydrates, sugar, soluble and insoluble fiber, sodium, vitamins, minerals, fatty acids, amino acids and more. Limes contain unique flavonoid compounds that have antioxidant and anti-cancer properties. These flavonoids have been shown to stop cell division in many cancer cell lines and are perhaps most interesting for their antibiotic effects (Tomotake, 2006). C. aurantifolia exhibits bioactive activities for colds, fevers, sore throats, sinusitis, bronchitis and asthma (Khan et al., 2012).

2. MATERIALS AND METHODS

Collection of plant materials:

Fresh B. pinnatum and C. aurantifolia leaves were obtained from home gardens in Benin City, Edo State, Nigeria and identified in Department of Plant Biology and Biotechnology of the University of Benin, Benin City, identification was confirmed with appropriate literature (Akobundu and Agyakwa, 1998; Keay, 1989). The leaves were air-dried, grinded and made into a fine powder using laboratory mortar and pestle and kept in a sterile air-tight container to avoid contamination.

Preparation of extract:

Fifty grammes each of dried pulverized leaf powder was dissolved in 500 ml each of distilled water (to make aqueous extract) for 24 hrs and centrifuged at 3000 rpm to enable paper diffusion of the active ingredients into the extraction medium. Filtration was later carried out using Whatman's (No. II) filter paper and the filtrate was evaporated to dryness using steam water-bath at 100 oC. This procedure was further carried out with ethanol, methanol and acetone to obtain ethanol extract, methanol extract and acetone extract respectively. The extracts were now stored at 4 oC in a refrigerator. Combination of both plants was used in the synergistic assessment.

Test Organisms:

Bacterial cultures of the test organisms, Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli and Pseudomonas aeruginosa were obtained from the Department of Medical Microbiology, University of Benin teaching hospital, Benin City, Nigeria. Their identity was confirmed using cultural, morphological and biochemical test as described by Cheesebrough et al. (2002). They were maintained on nutrient agar slants at 4 0C. These test bacteria have

been previously described (Prescott et al., 2008 Akinnibosun et al., 2008a, b). Fungal species from laboratory stock of the Department of Microbiology, University of Benin, Benin City, identified and characterized based on their morphological characteristics and microscopic analysis by using taxonomic guides and standard procedures (Gilman, 1944; Barnett and Hunter, 1972; Ellis, 1976; Domsch et al., 1980), were also used as test organisms (Aspergillus niger, Mucor mucedo, Penicillium notatum and Candida albicans). Phytochemical screening of the extracts: Phytochemical screening of the extracts was carried out according to methods described by Odebiyi and Sofowora, (1978) and Trease and Evans, (1989) as follows: Test for Alkaloids: Five grams of evaporated extract was boiled with 5 ml of 2 % HCL on a steam bath for 5 mins, the mixture was filtered after cooling, and the filtrate was shared into 3 test tubes A B and C. 1 ml portion of filtrate was treated with 2 drops of Mayer's reagent, a creamy white precipitate was observed. To confirm this result, 1 ml portion of the filtrate was treated with Dragendoff's reagent which gave a red precipitate to indicate the presence of alkaloids. Test for Flavonoids: Five grams of extracts was introduced into a test-tube containing 10 ml ethyl acetate solution and heated in boiling water for 1 min, the mixture was filtered and 4 ml of filtrate was shaken with 1ml of 1 % aluminum chloride solution and left to stand for 10 mins. The formation of a yellow colouration in the presence of 1 ml of dilute ammonia solution, indicated the presence of flavonoids.

Test for Saponins:

One gram of extract was boiled with 5ml of distilled water for 5 mins and the mixture was filtered while hot. To 1 ml of filtrate, two (2) drops of olive oil was added, the mixture was shaken and observed for the formation of emulsion, then 1 ml of the filtrate was diluted with 4ml of distilled water. The mixture was shaken and observed for the formation of stable frothing on standing, which indicated positive for saponins. Test for Tannins: To 2 g of the sample, 5 ml of 45 % ethanol was added and boiled for 5 mins. The mixture was cooled and filtered. To 1 ml of the filtrate, three (3) drops of lead acetate solution was added. The formation of gelatinous precipitate indicates the presence of tannins. Also as a confirmation test, 1 ml of filtrate was treated with 0.5 ml of bromine water and the formation of a pale brown precipitate indicates the presence of tannins. Test for glycosides: Two grams of samples were mixed with 30 ml of distilled water and boiled for 5 mins in a water bath. The mixture was cooled and filtered. To 5 ml of the filtrate, 0.2 ml of Fehling's solution A and B were added and boiled further in a water bath for 2 mins. A brick red colouration which indicates the presence of glycosides was noticed. Test for Reducing Sugar: About 5 g each of the dried samples was introduced into a test tube and equal amount of Fehling's solution A

and B were added. The mixture was boiled over a burner and observed for colour change. The colour changed from deep blue to brick red, indicating the presence of reducing sugar. Test for Steroids: About 2 ml each of concentrated sulphuric acid (H2SO4) and acetic anhydride were poured into 5ml each of the aqueous extract samples. The colour changed from violet indicates the presence of steroids.

Determination of Antimicrobial Activity:

The crude extracts were screened for antimicrobial activity by determining the zone of inhibition against the test organisms using agar-well diffusion method. Sterile Mueller-Hinton agar plates were inoculated with prepared inoculum with sterile cotton swab. Then with the help of sterile cork borer, wells were made in the inoculated media plate. 50 µl of the working solution/ suspension of different concentration were transferred into the well with the help of micropipette. The control was also placed in the separate well at the same time. After proper incubation, the plates were viewed for the zone of inhibition, which is suggested by clear areas without growth around the well.

3. RESULTS AND DISCUSSION

Microbial resistance to several antibiotics is becoming a source of challenge and concern to public health. In view of the increasing rate of antimicrobial drug resistance ravaging not only the African continent but the world at large, alternative, effective and affordable substitutes are essential if bacterial infections are to be properly controlled. Phytochemicals are secondary metabolites of plants known to exhibit diverse pharmacological and biochemical effects on living organisms. Many plants containing alkaloids and flavonoids have diuretic, anti-inflammatory and analgesic effects. Alkaloids are capable of reducing headache associated with hypertension. It has been reported that alkaloids can be used in the management of cold, fever and chronic Catarrh.

Flavonoids are known for their antioxidant activity and hence they help to protect the body against cancer and other degenerative diseases (Jindal et al. 2012). Tannins are known to exhibit antiviral, antibacterial and antitumor activities. Saponin is used as hypercholesterolemia, hyperglycaemia, antioxidant, anticancer, anti-inflammatory and weight loss. The presence of these phytochemicals (steroids, tannins, reducing sugars, flavonoids, alkaloids, saponins and cardiac glycosides) in B. pinnatum and C. aurantifolia used in this study (Tables 1 – 3) supports their use as medicinal plants. These chemical constituents could be responsible for their antibacterial activity (Gill, 1992).

Different plant parts contain a complex of chemicals with unique biological activity (Farnsworth and Bingel, 1977), which is thought to be due to toxins and secondary metabolites, which act as attractants or deterrents (Fisher, 1991). Over the years, these bioactive principles have been exploited in tradomedical practice for the treatment of various ailments (Adebanjo et al., 1983). Antimicrobial resistance of pathogenic bacteria to current synthetic drugs has necessitated the investigation into new, safe, efficient and costeffective antimicrobial agents as alternative agents for controlling the infectious diseases (Khan et al., 2012). The extent of sensitivity of the test organisms to the plant fractions was assessed by measuring the zone of inhibition after 24 hrs incubation. Table 4 shows the antimicrobial activity of B. pinnatum leaf extract using different extracting solvents. The results revealed that the ethanol extract of B. pinnatum was most effective against the test organisms than the other extracting solvents. S. aureus showed the highest susceptibility (17.3 ± 1.2 mm) to B. pinnatum ethanol extract, while P. aeruginosa showed the least susceptibility (8.3 ± 0.9 mm). The results also revealed that all the test fungi were resistant to the different extracts except C. albicans. The ethanol extract of B. pinnatum was the most effective against the C. albicans, while acetone extract was the least effective compared to the other extracting solvents. This is in agreement with the observations of Ammara et al., 2009, who concluded that the stronger extraction capacity of ethanol could have been responsible for the higher antimicrobial activity. Table 5 shows antimicrobial activity of C. aurantifolia leaf extract using different extracting solvents. The results revealed that the methanol extract of C. aurantifolia was most effective against the test organisms than the other extracting solvents. This explains the reason for the highest antimicrobial activity of C. aurantifolia using methanol as the extracting medium. The stronger extraction capacity of methanol for C. aurantifolia could have been responsible for the higher antifungal activity. The biologically active components in the plant could have been enhanced in the presence of methanol (Tshesche, 1970). C. albicans showed the highest susceptibility (18.7 ± 0.9 mm) to C. aurantifolia methanol extract, while P. notatum showed the least susceptibility to methanol extract (8.0 ± 0.9 mm). The aqueous extract had the least effect on the test organisms, followed by the acetone extract. Acetone extract of C. aurantifolia was only effective against C. albicans (11.7± 0.9 mm). S. aureus showed the highest susceptibility (25.3 ± 0.9 mm) to C. aurantifolia ethanol extract, while P. aeruginosa showed the least susceptibility (12.7 ± 0.9 mm).

4. CONCLUSION

This study has shown that combinations of extracts demonstrated synergistic and additive effects on microorganisms. The synergy is better, as microbial tolerance is less likely to develop against substances having more than one type of mode of action. Differential antimicrobial activity of the extracts against different bacteria and fungi was due to the presence of different active phyto-compounds which made the test organisms to be susceptible. It is therefore recommended that the synergistic use of medicinal plant extracts be encouraged to prevent drug resistance and treat the emerging and reemerging diseases caused by the bacterial and fungal species.

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A FIELD PROJECT REPORT ON

PHYTOREMEDIATION OF PETROLEUM HYDROCARBONS

Submitted in fulfilment of the requirements for the award of the degree of

BACHELOR TECHNOLOGY

In

BIOTECHNOLOGY

Submitted By

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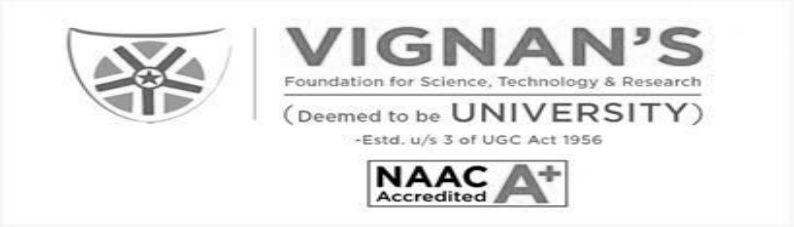

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January-2023

VIGNAN'S
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NAAC Accredited A+

CERTIFICATE

This is to certify that the field project entitled "PHYTOREMEDIATION OF PETROLEUM HYDROCARBONS" being submitted by Mandalapu Geethanjali (211FA14048), Cherukuri Madhusree (211FA14049), Naidu Likhitha Priya (211FA14050), Hari Priya Narra (211FA14051), Meda Naga Anjana Gopinath (211FA14052), Ajay Chowdary Yarramaneni (211FA14053) in the partial fulfilment of Bachelor of Technology project in the department of BIOTECHNOLOGY, Vignan's Foundation for the science technology and Research, Vadlamudi, Guntur District, Andhra Pradesh, India, is a bonafide work carried out by them under our guidance and supervision.

Dr. N. Jalaja
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Prof. T.C. Venkateswarulu
HOD Biotechnology

DECLARATION

We hereby declare that our project work described in the field project titled "PHYTOREMEDIATION OF PETROLEUM HYDROCARBONS" which is being submitted by us for the partial fulfilment of the in the department of Biotechnology, Vignan's Foundation for Science, Technology and Research (Deemed to be University), Vadlamudi, Guntur, Andhra Pradesh, and the result of investigations are carried out by us under the guidance of Dr. N.Jalaja

Mandalapu Geethanjali (211FA14048)
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CONTENTS

1. INTRODUCTION

Many developing nations face serious soil contamination problem. As a result of human economic activity, large quantities of soil have been contaminated with petroleum products (Kaimi et al, 2006). Petroleum contaminated soil causes pollution of local ground water by organics, threatens the safety of potable water, limits the use of ground water, causes enormous economic loss and ecological disaster, and destroys agricultural production (Wang et al, 2007; Xu et al, 2006). Soil pollution has become an environmental problem that affects human beings (Escalante-Espinosa et al, 2005). Remediation of oil polluted soil has attracted worldwide attention. Phytoremediation is regarded as a cost-effective method for removal of petroleum from soil, and has a great potential in remediation of soil contaminated with petroleum (Joner et al, 2002; Ryan et al, 2001). In addition, this method is not destructive and could remedy the soil structure and recover the biological environment (Maria et al, 2002). Phytoremediation of polluted soil has been applied successfully. Because of their sessile nature, plants develop the ability to resist a wide range of environmental assaults, plants enhance the remediation of petroleum-containing soils by various processes, including elimination, destruction or sequestering hazardous substances from the environment (Maila and Cloete, 2002; Vervaeke et al, 2003). Laboratory and pot experiments demonstrated that plants enhanced the dissipation of poly-aromatic hydrocarbons (PAHs) when compared to unplanted controls (Joner et al, 2002; Chen et al, 2003). Phytoremediation eld trials showed that the reduction of petroleum hydrocarbons in the rhizosphere was accelerated (Chen et al, 2003; Glick, 2003). Liste and Alexander (2000) reported that the degradation of pyrene can be promoted by nine different plant species, including three field crops, three horticultural plants, and three pine seedlings. The key issue for successful phytoremediation was the application of plant species that have the ability to proliferate in highly contaminated soil. The aim of the present study was to investigate the effect of three different plant species on microbial remediation of petroleum polluted soil. The contaminated soil used in this study was from Dagang Oil Field, and Pannicum, Eleusine indica (L.) Gaerth, and Tall Fescue were selected for remediation of the petroleum polluted soil. The changes of microbial number, petroleum content, dehydrogenase activity, catalase activity in the soil and the moisture of the soil were monitored regularly during the pot experiment. The possible factors for these changes are discussed in this paper. The influence on the degradation of hydrocarbons in petroleum polluted soil is presented, providing a theoretical and technical support for phytoremediation of petroleum polluted soil.

2. PROBLEM STATEMENT

An experimental study of the rhizosphere effect on phytoremediation of petroleum polluted soil was carried out with three species of grasses, namely Pannicum, Eleusine indica (L.) Gaerth, and Tall Fescue. After a period of 150 days, this pot experiment showed that the rhizosphere of these three species accelerated the degradation of petroleum hydrocarbons to different extents. The results showed that the number of microorganisms in the rhizosphere increased by three orders of magnitude. The induction of the plant rhizosphere and the coercion influence of petroleum changed the species and activity of microorganisms. The degradation of petroleum hydrocarbons in the rhizosphere was 3-4 times that in unplanted soil. The dehydrogenase activity in the rhizosphere was 1.61-2.20 times that in unplanted soil, but the catalase activity was 0.90-0.93 times that in unplanted soil, and soil moisture content increased by 5% compared with the unplanted soil.

3. REQUIREMENTS

3.1 MATERIALS REQUIRED

- Sand 7.8% (by weight),
- Heavy clay 5.4% (by weight) and
- Oil content 8,247 mg·kg-1.
- Water
- Soxhlet
- Glass electrode apparatus (Sartorius PB-10)
- Petri plates
- Colony counter
- Spectophotometer

3.2 CHEMICALS REQUIRED

- CCl4 as solvent
- Tester OIL-20A
- organic carbon 72.5 mg·kg-1, total N 4.15 mg·kg-1, total P 0.42 mg·kg-1
- Potassium dichromate
- Sodium hydroxide
- Peptone
- Beef-extract
- Agar
- Triphenyl tetrazolium chloride
- KMnO4

4. PROJECT DESCRIPTION

Polluted soil for experiment:

The petroleum polluted soil was from Dagang Oil Field, Bohai Bay Basin, East China. The soil was sieved with a 5mm-nylon screen after solar drying. The characteristics of the soil are as follows: pH 8.9, total organic carbon 72.5 mg·kg-1, total N 4.15 mg·kg-1, total P 0.42 mg·kg-1, sand 7.8% (by weight), heavy clay 5.4% (by weight) and oil content 8,247 mg·kg-1. \

Plant for remediation of petroleum polluted soil:

Three grass species, Pannicum, Eleusine indica (L.) Gaerth, and Tall Fescue, were selected for phytoremediation. Tall Fescue was used for phytoremediation and showed to be more tolerant of petroleum than other tested species in our previous work. Pannicum and Eleusine indica (L.) Gaerth were new tested species.

Experimental procedures:

First, the petroleum polluted soil was mixed fully with pollutant-free soil to reduce the oil content to 5,000 mg·kg-1. The experimental pot had a height of 33 cm and a diameter of 23 cm. Each pot contained 12 kg soil. At the beginning of experiment, the soil in the pot was irrigated until it was saturated. The pot experiment was conducted outdoors in August and September. From October to December, the experiment was conducted indoors. Two grams of seeds were soaked separately in 75% (v/v) ethanol for 30 minutes. Then, the seeds were washed with clear water then soaked in clear water for 3-4 days. When the white radicles appeared, the seeds were transplanted to the pot. During the experiment period, the irrigation frequency and the volume of irrigation water were controlled to prevent petroleum in the soil from washing out. The irrigation interval was once every two days, the volume of irrigation water was 50 mL for each pot. Simultaneously an unplanted pot was used as a control soil and operated in the same procedures.

Chemical analysis:

Soil was collected for analysis at 0, 30, 60, 90, 120 and 150 days. The sample soil was taken from a depth of 7 cm under ground near the root, according to the plum blossom method (He, 2001). Five samples were taken for analysis at one time. Soil samples were analyzed for

moisture, dehydrogenase activity, catalase activity, microbial number and petroleum content.

Measurement of petroleum content of soil:

The petroleum content of the soil was measured with the Soxhlet method (US EPA, 1996). First, the soil was dried under vacuum. Then petroleum was extracted from a 5 g sample of soil using CCl4 as solvent for 6-8 hours until CCl4 became colorless. The extract was diluted with four parts of CCl4 to 1/5. Finally, the diluent was injected into an oil content tester OIL-20A to determine the petroleum content. 2.4.2 Measurement of characteristics of soil The moisture content of soil was measured by the loss of water at 40 ºC and -0.08MPa; The pH of soil was measured by a glass electrode apparatus (Sartorius PB-10). The total organic carbon of soil was measured with the potassium dichromate method, the total N with the Kjeldahl method and the total P with sodium hydroxide digestion method (Liu, 2001).

Measurement of microbial levels in rhizosphere soil:

The microbial population was measured with the spread plate counting method. The incubation media used for isolation of bacteria was peptone, beef-extract and agar. Plates were incubated for 3 days at 35 ºC prior to counting the colony forming-units (cfu).

Enzyme activity in rhizosphere soil:

The dehydrogenase activity in the rhizosphere soil was determined according to the triphenyl tetrazolium chloride method (Hayano, 1997). For this, 1 g of the soil was cultivated in 0.2mL of 0.4% triphenyl tetrazolium chloride solution with 50 l of 1% glucose for 24 h at 27 ºC in a dark environment. The TF (triphenyl formazan) formed by enzyme reactions was extracted by using 10 mL of methanol, shaken vigorously for 1 minute, and then ltered. Triphenyl formazan was measured spectrophotometrically at 486 nm. The catalase activity was determined according to the potassium permanganate method (Guang, 1986). For this, 1 g of the soil was titrated using KMnO4 solution at a concentration of 1×10-3mol·L-1.

5. WORKING

5.1 Change of microbial activity:

In the phytoremediation process, the microbial population in the rhizosphere soil was measured. The microbial numbers in planted and unplanted soils were measured by the most probable number method.

5.2 Measurement of petroleum content of soil:

The petroleum content of the soil was measured with the Soxhlet method (US EPA, 1996). First, the soil was dried under vacuum. Then petroleum was extracted from a 5 g sample of soil using CCl_4 as solvent for 6-8 hours until CCl_4 became colorless. The extract was diluted with four parts of CCl_4 to 1/5. Finally, the diluent was injected into an oil content tester OIL-20A to determine the petroleum content.

5.3 Measurement of characteristics of soil

The moisture content of soil was measured by the loss of water at 40 ºC and -0.08MPa; The pH of soil was measured by a glass electrode apparatus (Sartorius PB-10). The total organic carbon of soil was measured with the potassium dichromate method, the total N with the Kjeldahl method and the total P with sodium hydroxide digestion method (Liu, 2001).

5.4Measurement of microbial levels in rhizosphere soil

The microbial population was measured with the spread plate counting method. The incubation media used for isolation of bacteria was peptone, beef-extract and agar

6 RESULTS & DISCUSSION

6.1 Change of microbial activity:

In the phytoremediation process, the microbial population in the rhizosphere soil was measured. The microbial numbers in planted and unplanted soils were measured by the most probable number method, and the results are shown in Fig. 1.

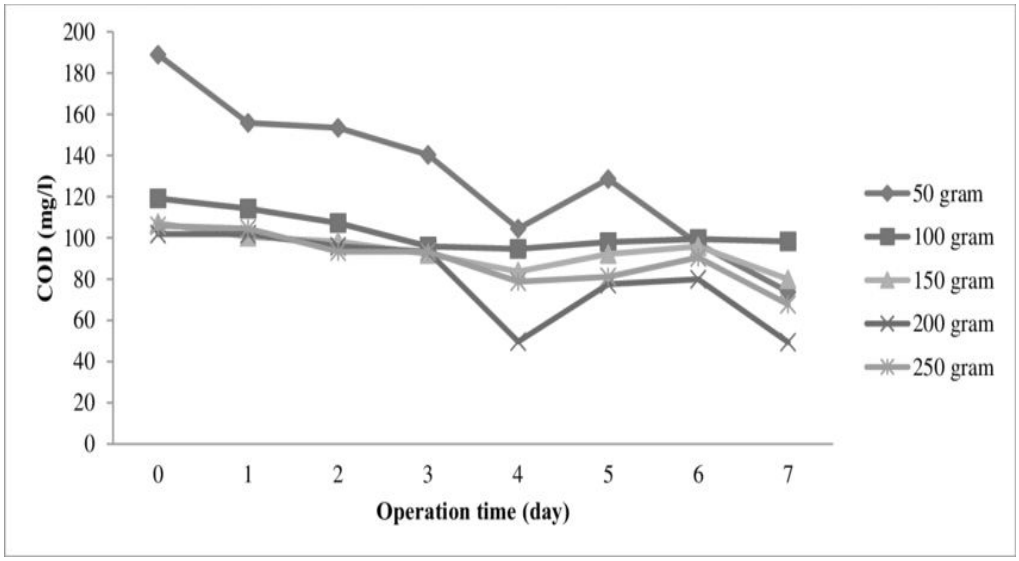

Fig. 1 shows that in the 150 days remediation process the number of microorganisms in the rhizosphere increased by three orders of magnitude compared to the unplanted soil, and the number of microorganisms increased by two orders of magnitude after 90 days of remediation. From 90 days to 150 days, the microbial number increased by one order of magnitude. The numbers of microbes were different between the earlier (from 0 to 90 days) and the later period (from 90 to 150 days). Except for the different intervals of measurement time, the reason might be that the secretion of plant roots in the earlier period was more than that in the later period. Plant roots release compounds including monosaccharides, amino acids, enzymes, aliphatics, and Fig. 1 Change of microbial number 0 90 150 0 1 2 3 4 5 6 7 8 log(Microbial number) Time, d Unplanted Pannicum Eleusine indica(L.) Gaerth Tall Fescue Pet.Sci.(2008)5:167-171 169 aromatics that stimulated the growth of specific microbial communities (Crarela et al, 2000).

5.3 Measurement of petroleum content of soil:

The petroleum content of the soil was measured with the Soxhlet method (US EPA, 1996). First, the soil was dried under vacuum. Then petroleum was extracted from a 5 g sample of soil

using CCl_4 as solvent for 6-8 hours until CCl_4 became colorless. The extract was diluted with four parts of CCl_4 to 1/5. Finally, the diluent was injected into an oil content tester OIL-20A to determine the petroleum content.

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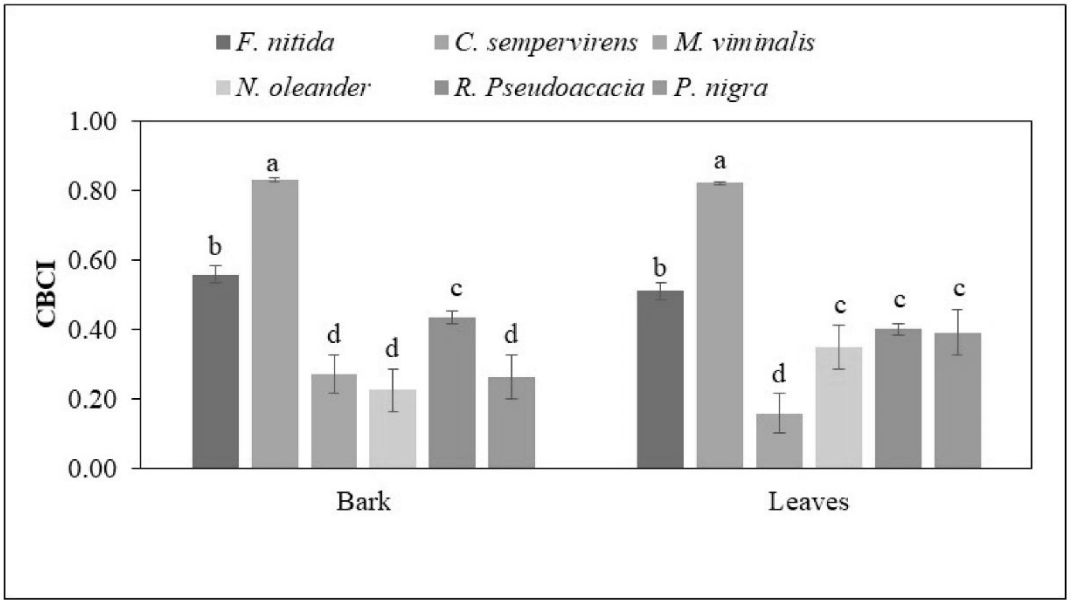

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6 CONCLUSION

Three species of plants, i.e. Pannicum, Eleusine indica(L) Gaerth, and Tall Fescue, selected for phytoremediation of petroleum polluted soil accelerated the degradation of petroleum hydrocarbons to different extents. Two new species, Pannicum and Eleusine indica (L.) Gaerth, had remarkable remediation effect similar to Tall Fescue. The number of microorganisms in the rhizosphere increased by three orders of magnitude. The action of the plant rhizosphere and the influence of petroleum hydrocarbons changed the species and activity of microorganisms. The degradation of petroleum hydrocarbons in the planted soil was 3-4 times that in the unplanted soil. The dehydrogenase activityin the planted soil

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A Field Project Report on

Custard Apple Seed Oil as A Pesticide

Submitted in fulfilment of the requirements for the award of the degree of

BACHELOR TECHNOLOGY

In

BIOINFORMATICS

Submitted By

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Under the guidance of

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January-2023

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This is to certify that the field project entitled "Custard Apple Seed Oil as A Pesticide" **being submitted by** 211FA14055 (MEDARAMETLA MEGHANA), 211FA14057 (BATTULA RAJENDRA), 211FA14058 (BONTHU YAMUNA), 211FA14059 (JAGARLAMUDI AMMANNI), 211FA14060 (POLINENI RAJYA LAKSHMI), in partial fulfillment of Bachelor of Technology project in the department of Biotechnology, Vignan's Foundation For Science Technology & Research (Deemed to be University), Vadlamudi, Guntur District, Andhra Pradesh, India, is a bonafide work carried out by them under guidance and supervision.

Dr. A. Venkata Narayana
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DECLARATION

.

We hereby declare that our project work described in this field project entitled "**Custard Apple Seed Oil as A Pesticide"** which is being submitted by us for the partial fulfillment of the current project in the department of Biotechnology**,** Vignan's Foundation for Science Technology & Research (Deemed to be University), Vadlamudi, Guntur District, Andhra Pradesh, India, and the results of investigation are carried out by us under the guidance of Dr. Dr. A. Venkata Narayana, Associate Professor.

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CONTENTS

1. INTRODUCTION

Maharashtra is the leading state in custard apple production with 92,320 tons. The raw material required for the process; custard apple seeds are available in abundance. There are various methods of custard apple seed oil extraction. The selection of appropriate solvent for extraction is done by taking into account various factors like cost, oil extraction efficiency, solvent recoverability, and environmental impacts.

2. MATERIALS AND METHODS

2.0 METHODS OF OIL EXTRACTION:

2.1 Conventional Methods

Conventionally, the oil for pesticides can be obtained by three methods:

1. Custard apple seeds are boiled in an approximate amount of water until the liquid is reduced to half. Dilute the filtrate with a high quantity of water to obtain the pesticide.
2. Add finely ground custard apple seeds to water. Leave it to stand for a day or two. Strain.
3. Grind seeds to extract oil using a grinder. Dilute one part of oil to 20 parts.

2.2 Experimental Methods

2.2.1 Cold Pressing

This method is most preferred for extracting oil from citric rinds such as orange, lemon, and grapefruit. This method involves the simple pressing of the rinds at a temperature of about 120 F to extract the oil. These rinds are first separated from the fruit, ground, or chopped and then are finally pressed. The result obtained is a watery mixture of oil and ethanol. The liquid which we separate given time little alteration from the oil original state occurs this citrus oil retain their bright, fresh, uplifting, aroma like that of smelling wonder cooling ripe fruit. The drawback of this method is the oil extracted using this has a short shelf life.

2.2.2 Solvent Extraction

In the solvent extraction method, the oil recovery and extracting unit is loaded with perforated trays of oil plant material and repeatedly wash with the solvent. A carbon and hydrogen-based solvent are used for extraction. All the extractable material from the plant is dissolved in the solvent. This includes highly volatile aroma molecules as well as non-aroma waxes and pigment. The extract is distilled to recover the solvent for future use. The waxy mass that remains is known as the concrete. The concentrate concrete is further processed to remove the waxy material which diluted pure oil. To prepare the absolute from the concrete

the waxy concrete is warm and stirred with alcohol (Ethanol). During the heating and stirring process, the concrete breaks up into min globules. Since the aroma molecule are more soluble in alcohol than the waxes and efficient separation of two results.

2.2.3 Steam Distillation

Steam distillation is a special type of distillation or a separation process for temperature-sensitive materials like oils, resins, and hydrocarbons, etc. which are insoluble in water and may compose at their boiling point. The fundamental nature of steam distillation is that it enables a compound or a mixture of the compound to be distilled at a temperature that contains a substance substantially below that of the boiling point of the individual constituents. Essential oils contain a substance with a boiling point up to 200°C or higher temperature. In the presence of steam or boiling water, however, the substances are volatilizing at a temperature of 10°C very close to atmospheric pressure. Various factors determine the final quality of a steam distilled essential oil. Apart from plant material, the most important are time, temperature and pressure, and quality of the distillation equipment. Essential oils are very complex products. Each is made up of many, sometimes hundreds, of distinct molecules which come together to form the oil's aroma and therapeutic properties. So of these molecules are fairly delicate structures that can be altered or destroyed by adverse environmental conditions so much like a fine metal is more flavourful that longer distillation times may give more complete oil. It is also possible, however, that longer distillation times may lead to the accumulation of more artifacts than normal. This may have a curious effect of appearing to improve the odor, as sometimes when materials that have a large number of components are sniffed, the perception is often of slightly increased sophisticated, added fullness and character, and possibly, and extra pleasantness.

2.2.4 Maceration

The simple widely used procedure involved leaving the pulverized plant to soak in suitable solvents in a closed container. Simple maceration is performed at room temperature by mixing the ground grub with the solvents and leaving the mixture for several days with occasional shaking or starring. The extract is then repeated from the plant particles by stirring. The process is repeated with a fresh batch of solvent at least two times. Finally, the last residue is pressed out of the plant particles using the mechanical press or centrifuge. Kinetic maceration is different from a simple one by continuous stirring. This method can be used for both initial and bulk extraction.

2.2.5 Percolation

The powdered plant material is socked initially in a solvent in a percolator. Additional solvent is then poured on the top of the plant material and is allowed to percolate slowly out of the bottom percolators. Additional filtration of the extract is not required because there is a filter at the percolator.

2.2.6 Tincture

A tincture is typically an alcoholic extract of plant or animal material or a solution of such or of a low volatility substance. To qualify as an alcoholic tincture extract, the extract should have an ethanol percentage of at least. Sometimes an alcohol concentration higher than 90% is used in tincture. Alcoholic tinctures are made with various ethanol concentrations are the most common.

3. SOLVENT SELECTION

N-hexane is considered to be the most efficient solvent when dealing with oil extraction processes. When n-hexane is used the color obtained is the favorable yellowish-light brown as compared to the dark woody brown color obtained on using methanol as a solvent. Methanol gives the second-best yield after n-hexane. The acid value obtained was 1.683 for n-hexane. The natural pesticide produced from custard apple seed oil proves itself efficient, advantageous, cheap, and safe to handle. Its recovery by using Hexane solvent is 19% than the methanol solvent is 10.5%. This pesticide material can be made easily available for every farmer throughout India without taking much more effort. This raw material will be very cheap which minimizes the total cost of processing along with solvent recovery. Many factors like oil extraction efficiency, environmental impacts, and the renewability of solvents must be considered while selecting the ideal solvent. Hexane is the preferred solvent for the extraction of oils from plant sources due to its low boiling temperature and easy recovery from the extract. Most oils are soluble in hexane too. The cost of n-hexane in laboratories is Rs.45 per liter.

4. EXPERIMENTAL PROCEDURE

Seed kernels crushed and grounded to powder. Then the powder which is obtained from crushing is mixed with hexane or methanol solvent to extract oil from seed kernels. While doing the extraction, the solvent is used in the ratio of 15ml/g of seed kernels powder, and extraction time was 3hr, 4hr, 4hr for two hexane, and one methanol solvent respectively. The temperature was maintained near about 65- 70 degrees Celsius by regulating the magnetic cum heater and stirrer. After extraction, the sample is filtered out to remove solid material as residue, and the filtrate is contained with oil extracted. This filtered sample is lead to vacuum

distillation for the first sample and simple distillation for the other two samples. Then after distillation solvents were distilled out while the oil extracted was remain in the distillation chamber. Then lastly the oil separated is analyzed for density, percent of oil, and acid value.

5. ANALYSIS AND APPLICATION OF PESTICIDE

The oil obtained above is tested for insecticidal properties by standard methods such as HPLC, Spectrophotometry, Polarography, and FTIR. After carrying out these standard tests, and analyzing the properties, the oil is applied to the target pests. For example, when applying mealybugs on a guava tree, preparation of the blank solution is carried out. The blank solution is formed by mixing 6 parts of labolene soap with 94 parts of water. To this blank solution, the required percentage of custard apple seed oil is added and sprayed on the pest attacked surface with a help of a spray gun.[16][17][18].

6. PESTICIDE TEST ON WHITE MEALY BUGS

An experiment was carried out to test the effect of the pesticide on white mealybugs by Sikdar D.C et al,2016 [1]. Oil solution of (blank 0.0%,0.15%,0.30%, and 0.75% was prepared by mixing with labolene soap solution and sprayed in one shoot on white mealybug present on the guava tree leaves surface affected by white mealy bugs. The numbers of the white mealy bugs left on the leaf's surface after spraying the pesticide solution are counted daily. Based on the data tabulated by them, at 0.75% concentration, the pesticide was most effective on the target pests. The number of mealy bugs decreases to zero at 0.75% concentration within 2 days. Thus, an oil solution of 0.75% is effective to keep away the pests.

7. FUTURE SCOPE

The project work is on how a pesticide is prepared from a discarded waste material, custard apple seeds. This pesticide can be used in place of toxic synthetic pesticides. A study on the overall feasibility and profitability of the process can be carried out. The available cheap raw material required for the process can lead to the development of this industry. This operation could be carried out on a small scale and could generate employment for skilled as well as unskilled labor.

8. CONCLUSION

The various methods of oil extraction were compared and solvent extraction came forward as the best method of oil extraction. The entire process of extracting oil from custard apple seeds takes place in three and half hours including the time required for cleaning. N-hexane is the most preferred solvent for the extraction process as it is available for a price of Rs.45/liter and provides recovery of about 19%. The three conventional methods have a low yield but

can be performed at home at a negligible cost. Oil at 0.75% concentration is ideal to keep pests away.

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This is to certify that the project work entitled "**Proteomic profiling of diverse bacterial species causing bacterial gastroenteritis",** is being submitted by **Guruvelli Vasavi (191FA14009)** for partial fulfilment for the award of Bachelor of Technology in Biotechnology/Bioinformatics to **Vignan`s Foundation for Science, Technology and Research (Deemed to be University)** during 2022-2023, is a record of work carried out by his/her under our supervision. The results embodied in this work have not been submitted to any other university or institute for the award of any degree or diploma.

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This is to certify that the project work entitled **"establishing relation on critical fungal group pathogens for therapeutic design",** is being submitted by **Pakalapati George Emmanuel (191FA14014)** for partial fulfilment for the award of Bachelor of Technology in Bioinformatics to **Vignan`s Foundation for Science, Technology and Research (Deemed to be University)** during 2022-2023, is a record of work carried out by his/her under our supervision. The results embodied in this work have not been submitted to any other university or institute for the award of any degree or diploma.

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This is to certify that the project work entitled "**Liver disease prediction using Machine Learning**", is being submitted by **P. Dheeraj sri chandu (191FA14015)** for partial fulfilment for the award of Bachelor of Technology in Bioinformatics to **Vignan`s Foundation for Science, Technology and Research (Deemed to be University)** during 2022-2023, is a record of work carried out by his under our supervision. The results embodied in this work have not been submitted to any other university or institute for the award of any degree or diploma.

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This is to certify that the project work entitled **"Plant disease detection using MobileNetV2",** is being submitted by **P.Ramesh (191FA14016)** for partial fulfilment for the award of Bachelor of Technology in Bioinformatics to **Vignan`s Foundation for Science, Technology and Research (Deemed to be University)** during 2022-2023, is a record of work carried out by his under our supervision. The results embodied in this work have not been submitted to any other university or institute for the award of any degree or diploma.

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This is to certify that the project work entitled **"COMPUTATIONAL PREDICTION OF BIOMARKERS FROM DIFFERENTIAL EXPRESSION OF RNA-SEQ FOR BREAST CANCER PDX MODEL TISSUE",** is being submitted by **MOUNIKA POOLA (191FA14017)** for partial fulfilment for the award of Bachelor of Technology in Bioinformatics to **Vignan`s Foundation for Science, Technology and Research (Deemed to be University)** during 2022-2023, is a record of work carried out by his/her under our supervision. The results embodied in this work have not been submitted to any other university or institute for the award of any degree or diploma.

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This is to certify that the project work entitled **"Crop Recommendation based on season and productivity",** is being submitted by **Samireddy Hyma (191FA14019)** for partial fulfilment for the award of Bachelor of Technology in Bioinformatics to **Vignan`s Foundation for Science, Technology and Research (Deemed to be University)** during 2022-2023, is a record of work carried out by her under our supervision. The results embodied in this work have not been submitted to any other university or institute for the award of any degree or diploma.

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This is to certify that the project work entitled **"Multiple Disease Prediction Using Machine Learning",** is being submitted by **Tallapragada Ramapriya (191FA14020)** for partial fulfilment for the award of Bachelor of Technology in Bioinformatics to **Vignan`s Foundation for Science, Technology and Research (Deemed to be University)** during 2022-2023, is a record of work carried out by her under our supervision. The results embodied in this work have not been submitted to any other university or institute for the award of any degree or diploma.

(Dr.M.Viswajit) (Mrs.A.N.Lakshmi)

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This is to certify that the project work entitled **"IDENTIFICATION OF GBNV RELATED BIOMARKER IN THRIPS PALMI"**, is being submitted by **VAJIHA (191FA14022)** for partial fulfillment for the award of Bachelor of Technology in Bioinformatics to **Vignan`s Foundation for Science, Technology and Research (Deemed to be University)** during 2022-2023, is a record of work carried out by his/her under our supervision. The results embodied in this work have not been submitted to any other university or institute for the award of any degree or diploma.

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This is to certify that the project work entitled **"An NGS & Interaction network driven approach for identifying DEGs in RA and SLE",** is being submitted by **Venkata Chathurya Yatham (191FA14026)** for partial fulfilment for the award of Bachelor of Technology in Biotechnology/Bioinformatics to **Vignan`s Foundation for Science, Technology and Research (Deemed to be University)** during 2022-2023, is a record of work carried out by his/her under our supervision. The results embodied in this work have not been submitted to any other university or institute for the award of any degree or diploma.

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This is to certify that the project work entitled "**BREAST CANCER PREDICTION USING MACHINE LEARNING**" that is being submitted by **Ms.Venumadhavi Pemmasani** in partial fulfillment for the award of **B.Tech. Degree in Bioinformatics** to the Vignan's Foundation for Science, Technology, and Research (Deemed to be University) during 2022-2023, is a record of work carried out by her under our guidance and supervision. The results embodied in this work have not been submitted to any other University or Institute for the award of any degree or diploma.

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This is to certify that the project work entitled **"CREDIT CARD FRAUD DETECTION USING MACHINE LEARNING",** is being submitted by **SUDEEP CHOWDARY RAVIPATI (191FA14031)** for partial fulfilment for the award of Bachelor of Technology in Biotechnology/Bioinformatics to **Vignan`s Foundation for Science, Technology and Research (Deemed to be University)** during 2022-2023, is a record of work carried out by his/her under our supervision. The results embodied in this work have not been submitted to any other university or institute for the award of any degree or diploma.

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This is to certify that the project work entitled **"IN SILICO PREDICTION AND ANALYSIS OF ANTI MICROBIAL PEPTIDES FROM HUMAN MILK COMPARITIVELY COW MILK AND BUFFALO MILK".** is being submitted by **K.THANUSHA (191FA14034)** for partial fulfilment for the award of Bachelor of Technology in Biotechnology/Bioinformatics to **Vignan`s Foundation for Science, Technology and Research (Deemed to be University)** during 2022-2023, is a record of work carried out by his/her under our supervision. The results embodied in this work have not been submitted to any other university or institute for the award of any degree or diploma.

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This is to certify that the project work entitled "ERpred: a web server for the prediction of subtype-specific estrogen receptor antagonists", is being submitted **by PAVITHRA AVULA (191FA14001)** for partial fulfilment for the award of Bachelor of Technology in Bioinformatics **to Vignan's Foundation for Science, Technology and Research (Deemed to be University)** during 2022-2023, is a record of work carried out by her under our supervision. The results embodied in this work have not been submitted to any other university or institute for the award of any degree or diploma.

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This is to certify that the project work entitled "**Unravelling of Novel and Potent Drug Targets against Bacterial UTI**", is being submitted by **CHUKKAPALLI HARIKA (191FA14003)** for partial fulfilment for the award of Bachelor of Technology in Bioinformatics to **Vignan's Foundation for Science, Technology and Research (Deemed to be University)** during 2022-2023, is a record of work carried out by her under our supervision. The results embodied in this work have not been submitted to any other university or institute for the award of any degree or diploma.

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CERTIFICATE

This is to certify that the project work entitled **"Target Screening Against Different Plasmodium Species",** is being submitted by **Pragathi Reddy Duvvuru(191FA14004)** for partial fulfilment for the award of Bachelor of Technology in Bioinformatics to **Vignan`s Foundation for Science, Technology and Research (Deemed to be University)** during 2022-2023, is a record of work carried out by her under our supervision. The results embodied in this work have not been submitted to any other university or institute for the award of any degree or diploma.

Project Guide

Coordinator - UG Projects

Head of the Department

Signature of External Examiner

Vijay Rajeev .A

23/5/23

Place: Guntur

Date: 23 May 2023