Evaluation of the Potential of *Ricinus communis L* (Castor) Seed as a Substrate for Biorefinery Applications

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Evaluation of the Potential of *Ricinus communis L* (Castor) Seed as a Substrate for Biorefinery Applications

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for the Degree of
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IN

MICROBIOLOGY

By
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Faculty of Biological Sciences
Quaid-I-Azam University
Islamabad, Pakistan

2023

DEDICATED

TO

MY FAMILY

Author's Declaration

The material contained in this thesis is my original work and I have not presented any part of this thesis/work elsewhere for any other degree.

Saba Jamshaid

Certificate

This thesis submitted by **Saba Jamshaid** is accepted in its present form by the Department of Microbiology, Quaid-I-Azam University, Islamabad, Pakistan; as satisfying the thesis requirements for the degree of Master of Philosophy in Microbiology.

Supervisor: _____

(Dr. Malik Badshah)

External Examiner: _____

(Dr. Khalid Mehmood)

Chairperson: _____

(Prof. Dr. Naeem Ali)

Dated: 08-12-2023

Certificate

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Chairperson: _____

(Prof. Dr. Naeem Ali)

Dated: _____

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List of Abbreviations

AMR: Antimicrobial resistance

AR: Antibiotic resistance

ATCC: American type culture collection

AV: Acid value

DMSO: Dimethyl sulfoxide

DPPH: 2, 2-diphenyl 1-picrylhydrazyl

EV: Ester value

FAME: Fatty acid methyl esters

FFA: Free fatty acids

FTIR: Fourier transform infrared radiation

g: Gram

Kg: kilogram

L: liter

M: molar

MDR: multidrug resistant

MHA: Mueller Hinton agar

MIC: minimum inhibitory concentration

ml: milliliter

rpm: revolution per minute

SDA: Sabouraud dextrose agar

SDB: Sabouraud dextrose broth

SV: Saponification value

TG: Triglycerides

DG: Diglycerides

MG: Monoglyceride

ZOI: Zone of inhibition

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Saba Jamshaid

Abstract

The world is facing numerous ecological crisis, and health issues, necessitating sustainable, renewable, environmentally friendly, and economically feasible biofuels and antimicrobial compounds. In the current study *R. communis L* seeds were used as a feedstock for biorefinery, aiming to produce biodiesel using chemical transesterification and to evaluate its antimicrobial potential. The identification of bioactive molecules as prospective, affordable pharmaceutical resources derived from herbal plants is one novel approach to controlling the resistant diseases. *R. communis L* is widely used and well-known as a treatment for various diseases in traditional medicine. Phytochemical screening of *R. communis L* seed oil and extracts were carried out using qualitative phytochemical analyses. Its phytochemical properties are due to a variety of beneficial substances, including flavonoids, saponins, steroids/terpenes, resins/balsams, alkaloids, glycosides, tannins, and phenols, which are all detected in it. FTIR analysis also confirmed different functional groups in oil and extracts. In the current study, the antimicrobial potential of *R. communis L* extracts and seed oil, against five gram-negative bacterial strains (*Escherichia coli, Pseudomonas aeruginosa, Enterobacter, Klebsiella pneumoniae,* and *Salmonella enterica*), three gram-positive strains (*Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis*) and three MDR strains (*Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Salmonella*) and five fungal clinical strains (*Candida albicans, Aspergillus flavus, Aspergillus niger, Fusarium oxysporum* and *Curvularia lunata*) and three phytopathogenic strains (*Aspergillus flavus, Fusarium* and *Penicillium chrysogenum*) were evaluated by agar well diffusion method. Ethyl acetate, and methanolic extracts of *R. communis L* showed high activity against all selected bacterial strains while against fungal strains these extracts showed high to low range of activities. Methyl acetate extract showed high to low range of values for bacterial strains while for fungal strains it showed high to moderate range. Oil only showed activities against *E. coli* and *Fusarium* and didn't show any activity against all other bacterial and fungal strains. Ethyl acetate, methyl acetate, methanolic, n-Hexane and chloroform showed a good range of MICs values. Maximum antioxidant percentage was showed by 500mg/ml concentration of all extracts and oil. Extracts and oil didn't show cytotoxicity on brine shrimps except chloroform which showed slight toxic effect. Extracted oil was utilized for biodiesel production through chemical transesterification. Five factors (Oil to methanol ratio,

Temperature, Agitation, Reaction time and Catalyst concentration) were statistically optimized through Plackett Burman design. The maximum volumetric yield of biodiesel was recorded 96%, with conditions: temperature 60^{o}C, oil to methanol ratio 1:15, catalyst concentration 0.50%, 900rpm, and reaction time 60 minutes. Fourier transform infrared (FTIR) spectroscopy confirmed the production of biodiesel. The current study demonstrates that the *R. communis L* plant is an excellent feedstock for biorefineries.

Chapter # 1
Introduction

Introduction

Energy, health, and environment are basic components for the survival on the earth and all these are interlinked to each other. The world ecology is currently going through several crisis, threats, and destructions. The two main ecological threats are:

- Energy crisis
- Antimicrobial resistance

Solid fuels, like coal and fossil fuel, cause significant health risks, including occupational hazards and air pollution. Poor access to clean energy and energy efficiency can pose health hazards (Smith et al., 2013). Antimicrobial resistance, on the other hand, is a significant global issue. Antibiotic resistance poses a severe threat, according to the World Health Organization. WHO report suggests 10 million deaths will occur per annum by 2050 that will affect the economy and status of living worldwide. Runs of AMR from hospitals and pharmaceutical companies can cause environmental pollution that will affect human and animal health. By using “One health” approach energy crisis and AMR problems can be resolved (UNEP, 2023).

Most of the world's needs for energy, transportation, fuel, and commodities come from the usage of current fossil fuels, which has led to several economic and environmental problems. The depletion of fossil fuel reserves, rising fuel costs, and greenhouse gas emissions, which ultimately cause global warming and climate change, are some of the primary problems. These terrifying risks have shifted scientists' attention towards the development of sustainable as well as renewable energy sources for biofuels and profitable biomass-based goods in the setting of biorefineries (Saini et al., 2019) (Keyuraphan et al., 2012). By combining multiple production techniques and technologies, biorefineries produce biofuels, sources of renewable energy, and value-added products from plant biomass in a sustainable manner (Moncada et al., 2015). Biomass, a renewable energy source, is created when plants and animals store solar energy. It consists of organic matter like waste from animals, plants, algae, crops, and woody plants. Biomass energy is produced from biomass, which can be classified into first and second-generation feedstocks. The debate over "feed versus fuel competition" has arisen due to the expansion of production of biofuel as well as value-added goods with edible feedstocks. Food shortages may result from producing biofuels and

bioproducts from edible feedstocks in emerging nations (Weldemichael & Assefa, 2016) (Pessoa et al., 2019).

Non-edible second-generation feedstocks are considered the best for biorefinery, as they do not directly impact the food chain supply. Biofuels are also being explored for biodiesel production. *Ricinus communis L* is a non-edible feedstock it is being used in this study. It has pharmaceutical and industrial properties (Zulqarnain et al., 2021).

Biodiesel is a fatty acid methyl ester. It is produced by chemical transesterification of monohydric alcohols with fats and oils. The growing demand for biodiesel has led to international trade, partly due to agricultural land growth. The social acceptability of biodiesel depends on sustainable raw materials, non-competition with food and feed production, and avoiding species extinction (Brahma et al., 2022) (Estevez et al., 2022).

Biodiesel is a yellow-to-dark brown liquid with a high boiling point of up to 200°C and low vapor pressure. It has less sulphur content than mineral diesel and don't have aromatic compounds. It has a flash point above 130°C and is not dangerous. Biodiesel has a stable reactivity, mild odour, and is insoluble in water. Studies show it can reduce greenhouse gas emissions by up to 65%, making it an ideal alternative fuel for ecologically sensitive areas (Goodrum, 2002). Molar ratio (Musa, 2016) (Keera, Sabagh, et al., 2018), reaction temperature (Leung & Guo, 2006), reaction time (Yaakob et al., 2013), catalyst concentration are key factors for biodiesel production (Keera, Sabagh, et al., 2018).

Biodiesel produced from castor seed oil, offering several unique characteristics that make it an appealing substitute for traditional fossil fuels. Its high viscosity, low sulfur content, and biodegradability make it an environmentally friendly, renewable alternative resource to fossil fuels. Its high viscosity improves lubricating qualities, making it a valuable ingredient for industrial production. Its solubility in alcohol results in better cold flow characteristics, making it suitable for use even at low temperatures.

Castor oil's high ricinoleic acid content, which is a special fatty acid, lowers sulfur dioxide emissions, improving engine performance and combustion (Keera, Sabagh, et al., 2018) (Aziz et al., 2016). Its biodegradability also contributes to reducing pollution and contamination in the environment (Keera, Sabagh, et al., 2018). Castor oil-based biodiesel emits less CO_2 and particulate matter than regular diesel, reducing greenhouse

gas emissions and encouraging a more environmentally friendly energy sector. It is compatible with most current diesel engines without requiring engine changes, perform well, has a higher cetane rating, increase engine durability, and require less maintenance, making pollution checks cost-effective (Demirbas, 2009) (Singh & Singh, 2010) and its lower energy content does not significantly impact engine power or fuel economy (Rao et al., 2017).

Blending capacity allows for customization of biodiesel with petroleum fuel, allowing for customization for specific climates and engine types. Castor biodiesel's unique qualities make it a promising contender for a greener and more sustainable energy future (Singh & Singh, 2010).

Pakistan's economy can benefit from switching to biodiesel, which reduce reliance on fossil fuels and degrade naturally. This self-sustaining system uses CO_2 from plant growth, making it a more sustainable option.

Antimicrobial resistance is another major issue for survival. Antimicrobial resistance (AMR) emerged after Alexander Fleming discovered penicillin in 1928, leading to the development of more effective antimicrobial medicines. The era of antibiotics has saved numerous lives and reduced morbidity and mortality. However, the emergence of antimicrobial resistance has reduced their usage. Antimicrobial resistance (AMR) happens naturally (Vavala et al., 2016). Key causes include bacterial penicillinase (McArthur et al., 2013), biofilm formation, efflux pumps (Høiby et al., 2010), horizontal gene transfer, transposon transfer (David M. Livermore,2000) (Bengtsson-Palme et al., 2018), (Davies and Davies, 2010), excessive use of antibiotic (McGowan, 1983), and antifungal compounds (Vavala et al., 2016) (Davies and Davies, 2010), and microbial biochemical mechanisms (Davies and Davies, 2010) (Martinez, 2014).

Commonly known microbes which has developed resistance are *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Serratia*, *Escherichia coli, Acinetobacter baumannii*, *Salmonella enteric*, *Staphylococcus aureus*, *Clostridium difficile* (Davies and Davies, 2010) (McGowan, 1983), *Candida albicans, Aspergillus* species, *Aspergillus fumigatus,* and *Fusarium* species (Wiederhold, 2017).

Humans are harmed by antimicrobial resistance (AMR) at any stage of life. Over 5 million deaths and at least 1.27 million deaths worldwide were attributed to

antimicrobial resistance in 2019 (Vasconcelos et al., 2021). Antibiotics are responsible for roughly 2.8 million infections in the US each year. According to the CDC's 2019 antibiotic Resistance (AR) risks assessment, antibiotic resistance is responsible for over 35,000 deaths. The most frequent cause of AMR is antibiotic misuse (Valero & Salmeron, 2003). The use of naturally occurring compounds such as probiotics, prebiotics, essential oils, organic acids, and medicinal plants as antimicrobial alternatives has increased because of antibiotic resistance (Vaillancourt et al., 2018).

Antibiotic resistance has led to a global focus on developing plant-based antimicrobial drugs (Ameya et al., 2017) (Saeed and Tariq, 2005) (Pramila et al., 2012). Around 80% of people worldwide use plant extracts for therapeutic purposes, with 80% of traditional medicines using plant extracts and their active components (McKay and Blumberg, 2006) (Mahendran and Rahman, 2020). Secondary metabolites found in plant leaves, seeds, oil, and stems have antimicrobial effects. Plants and herbs offer alternatives to conventional drugs and therapies, with 50,000 plant species producing different compounds. Plant-derived medicines are cheaper, less susceptible to resistance, and have no side effects. Secondary plant metabolites have been extensively studied for therapeutic reagents, with antibacterial and antifungal properties (Anumudu et al., 2019) (Al-Lahham et al., 2020), (Pramila et al., 2012) (Capdesuñer et al., 2019).

The castor plant, scientifically called *Ricinus communis L*, belongs to family *Euphorbiaceae*. It has various common and dialectal names in different countries (Bueno et al., 2017). It grows in tropical and subtropical dry areas, with an annual, biennial, or perennial life cycle. Its germination period is from autumn to spring, with seed production lasting 6-7 months. In frosty areas, it acts as an annual crop (Gad et al., 2018) (M. Das et al., 2018).

It can germinate on undeveloped as well as marginal land. Its cultivation cost is low, and it has higher oil yields. Castor seed approx. 95% used for oil extraction. It is mainly composed of non-drying, ricinoleic acid triglycerides (Sundus et al., 2017).

Castor is cultivated in 30 countries for oil production, producing 220,000 tons annually. India contributes 70% of the export (Panhwar et al., 2016). In 2018, its global production increased by 1.8 million tons (Attia et al., 2018). Pakistan's oil seed production decreased from 1972 to 2021 reaching a peak of 3,795 tons in 2021 (knoema, 2022).

Numerous substances, such as triterpenoids, saponins, resins, flavonoids, lignin, tannins, alkaloids, phenolic compounds, and glycosides, have been found in *Ricinus communis*. Alkaloids are among the most significant of these substances. Its main components are found in the leaves, roots, and seeds (Aziz et al., 2016). Castor has antimicrobial, antioxidant, antinociceptive, antiasthmatic, antihistaminic, antifertility, immune system modulatory, hepatoprotective, anti-inflammatory, and wound healing activities (Jena & Gupta, 2012).

Aim and Objectives

Aim and objectives of the study

Aim

The aim of this study is to determine the antimicrobial potential of Methanolic, n-Hexane, Chloroform, Methyl acetate & Ethyl acetate extracts of *Ricinus communis L* (Castor) seed cake, and Biodiesel production by chemical transesterification.

Objectives

The objectives of this study are to:

- Identify *Ricinus communis L* seeds, extract oil and calculate its yield.
- Prepare Extracts to determine the antimicrobial potential of *Ricinus communis L* against selected microbial strains.
- Determine the antioxidant activity of *Ricinus communis L* seed cake extracts and oil.
- FTIR and Phytochemical analysis of *Ricinus communis L* oil and extracts.
- Evaluate the biodiesel production efficacy using *Ricinus communis L* through chemical trans-esterification reaction.
- Optimization of affecting parameters for biodiesel production by using Plackett-Burman design.

Chapter # 2
Literature Review

Literature Review

Energy, health, and environment are basic components for the survival on the earth. The world ecology is currently going through several crises, threats, and destructions. The main ecological threats are:

- Energy crisis
- Antimicrobial resistance

According to (Keyuraphan et al., 2012) (J. Saini et al., 2019) fossil fuel-based refineries are essential for the production of energy and several other industrial products, which are crucial to economic growth and considered as significant components of life. Fossil fuel supplies, on the other hand, are running out more quickly, and their consumption is a key cause of global warming as they contribute to environmental pollutants like CO_2 emissions. Therefore, using renewable energy sources like biofuels can help with the energy issue, especially in nations like Pakistan who are experiencing an oil scarcity (Zulqarnain et al., 2021). The following graph (Fig 2.1) depicts the primary energy consumption by fuels in 2021.

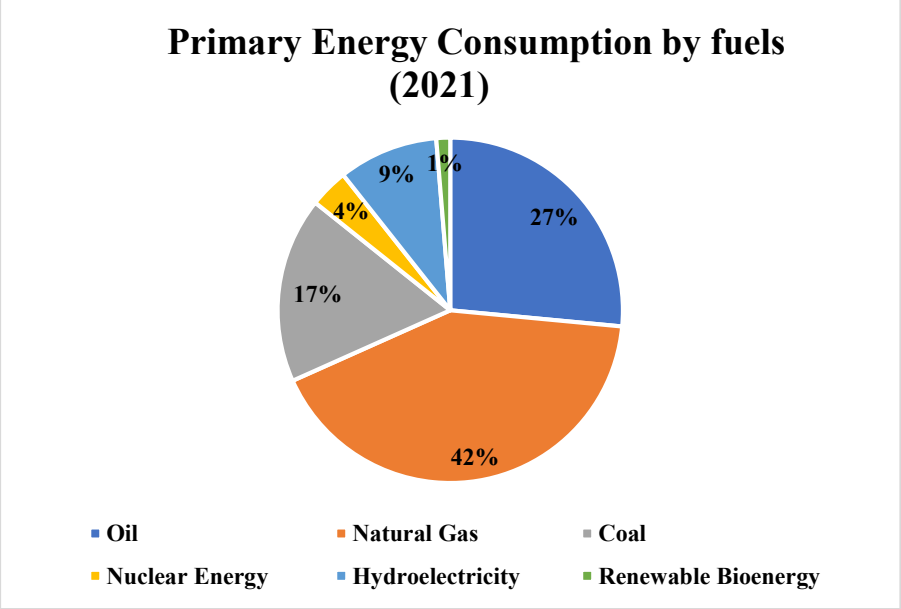


Fig. 2.1. Primary Energy Consumption in Pakistan by Fuels

(Statistical Review of World Energy, Energy economics, 2023)

2.1. Fossil Fuels and climate change

Climate change as well as global warming are the main concerns of the world nowadays. Countries are trying hard to solve these problems. Carbon dioxide emission occurs due to burning of fossil fuels and its emission reduction is the major issue to solve the global warming (Balasubramanian, 2014). In addition, compression ignition engines have released more dangerous air pollutants like gases e.g. sulfur dioxide, nitrogen oxides (NO_x), and particulate matter (Gopinath et al., 2015).

The usage of fossil fuels results in the indirect emission of hazardous gases, which contributes to climate change, melting of glaciers, rise in sea level, decline in biodiversity (Agarwal, 2007) (Amoah et al., 2019) (Keera, Sabagh, et al., 2018) and have adverse effect on environment and human health (Chidambaranathan & Seenikannan, 2014).

2.2. Rise in Crude oil Prices

The primary cause of a spike in crude oil prices, which ultimately has an effect on the worldwide economy, is an increase in consumer demand for fossil fuels for daily use, especially for industries and transportation (Agarwal, 2007) (Amoah et al., 2019). Transportation, the expansion of modernity, industrialization, and damaging environmental contamination have all contributed to an increase in global energy demand (Chidambaranathan et al., 2020).

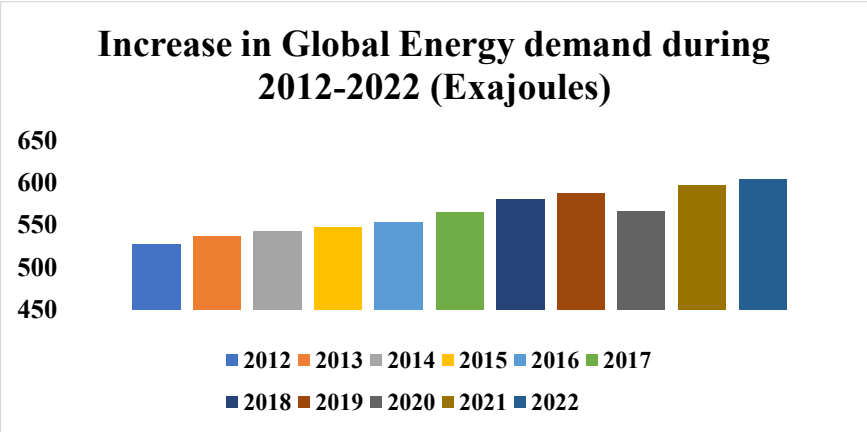


Fig. 2.2. Increase in Global Energy demand during 2012-2022

(Statistical Review of World Energy, Energy economics, 2023)

2.3. Pakistan's energy demand

Pakistan has been facing a severe energy crisis due to increasing demand and a focus on sustainable energy sources. The country's energy mix 2015 shows extensive dependence on traditional fossil fuels, with limited renewables. The country's primary energy demands are expected to reach 142 MTOE by 2025, increasing dependence on imported petroleum products. Improving energy efficiency and implementing energy preservation policies can help curtail imports and improve economic growth. Biomass based fuels are the best option for the countries like Pakistan (Ali et al., 2020).

2.4. Biomass based biofuels

Biofuel and other value-added chemicals production utilizing biomass in the setting of biorefineries is an optimal and cost-effective source from the social, economic, and environmental aspects, despite the emergence of a few alternatives in the previous few decades. According to (Weldemichael & Assefa, 2016) and (Pessoa et al., 2019), the use of biomass to produce biofuels and other value-added compounds can reduce the need for refineries. In the context of biorefineries, biomass has fewer advantages over fossil fuels.

- Biofuels are naturally biodegradable and other related products are readily available from biomass, as well as being socially and environmentally friendly.
- It is anticipated that they will soon account for a significant portion of the world's fuel market as biorefineries grow (Demirbas, 2009) (Keera, Sabagh, et al., 2018).

This fact has sparked a lengthy discussion on the viability and future of diesel engines, along with current criticism of emissions from diesel and persistent rumors of manufacturer deception (Chidambaranathan et al., 2020).

With additional advancements in engine technology and use of alternative fuels, hazardous emissions could be reduced. Increasing the variety of energy sources is also necessary to achieve low CO_2 emission levels (Ajiskrishnan et al., 2015). The world has also long been concerned about the prospect of fossil resources running out owing to careless consumption.

Furthermore, the imminent depletion of oil reserves and the rising social concern about the environment have spurred the development of sustainable and alternatives to traditional fuels that are affordable and sustainable (Chidambaranathan, et al., 2020). People have been driven to hunt for new sources of energy due to the depletion of petroleum supplies and the rise in price. For all these reasons, it is critical that nations consider the use of fuels that are renewable, sustainable, and economically viable. As a result, there has been an increase in interest in biofuels like biodiesel for applications involving diesel engines globally (Venugopal et al., 2017) (Keera, Sabagh, et al., 2018). Due to their high thermal efficiency and powerful output, diesel engines are becoming more desirable (Chidambaranathan & Kumar, 2018).

A range of feedstocks, such as used cooking and vegetable oil, animal fats, and algal oils, can be used to produce biodiesel (Chidambaranathan & Kumar, 2018). Since edible vegetable oils make up over 95% of the world's biodiesel production, they are seen as a possible diesel replacement (Bibin et al., 2020). However, for biodiesel industry, non-edible oils constitute a key source of raw materials. Most of the countries still employ edible oils in their agricultural outputs to cut costs and prevent the conflict between food and fuel (Bibin, 2019) (Chidambaranathan et al., 2020) (Demirbas, 2009).

For biodiesel synthesis rapeseed, sunflower, soya, and palm oils were identified as significant edible feedstocks after a global assessment of the possible feedstocks (Bibin et al., 2019). However, due to its numerous benefits, castor oil is a viable oil for the global biodiesel sector (Senthilkumar & Bibin, 2019). Biodiesel production from castor oil for commercial use has not been verified. However, studies of the available literature indicate that castor oil biodiesel production has lately increased (Senthil Kumar & Femina Carolin, 2019). Castor oil may be the top choice in terms of fuel properties, sustainability, and cost-effective feedstock for biodiesel fuel production (Valente et al., 2010).

2.5. Biodiesel

Chemically biodiesel is a fatty acid methyl ester and is used in much the same way as mineral diesel fuel. Chemical industry produce biodiesel by transesterification of monohydric alcohols like methanol or ethanol with vegetable or animal fats and oils.

Biodiesel has so far contributed the most in transport sector in the European Union. There was widespread social agreement to introduce and increase the supply of biodiesel toward the end of the 20th century. Growing demand over time has resulted in international trade in biodiesel, which was in part related to the growth of agricultural land, such as through slash-and-burn practices. The availability of raw materials that are sustainable, don't compete with the production of food and feed, or do not cause the extinction of species will determine whether a broad use is socially acceptable (Brahma et al., 2022).

2.5.1. Biodiesel production

Long chain alkyl fatty acid esters, also referred to as biodiesel, produced via combining triglycerides with alcohol (methanol or ethanol) in the presence of a catalyst. This reaction might be alkaline, acidic, or enzymatic. The three reversible phases that make up the transesterification procedure are as follows:

1. **Triglyceride to diglyceride conversion**

   TG+ ROH + Catalyst ⟹ DG (Diglycerides) + RCO_2R

2. **Diglyceride to monoglyceride conversion**

   DG + ROH + Catalyst ⟹ MG (Monoglycerides) +RCO_2R

3. **Glycerine synthesis**

   MG + ROH + Catalyst ⟹ RCO_2R (Ester) + GL (Glycerol)

Oil + Alcohol + Catalyst → Biodiesel + Glycerin

(Vilas Bôas et al., 2022)

2.5.2. Feedstocks used for Biodiesel production

According to Figure 2.3, the five categories of potential feedstocks for biodiesel production are fossil fuel; first-generation edible oil crops; second-generation non-edible crops; third-generation waste materials; fourth-generation algae-based crops and fifth generation genetically modified crops (ACS Omega 2021) (Zulqarnain et al., 2021).

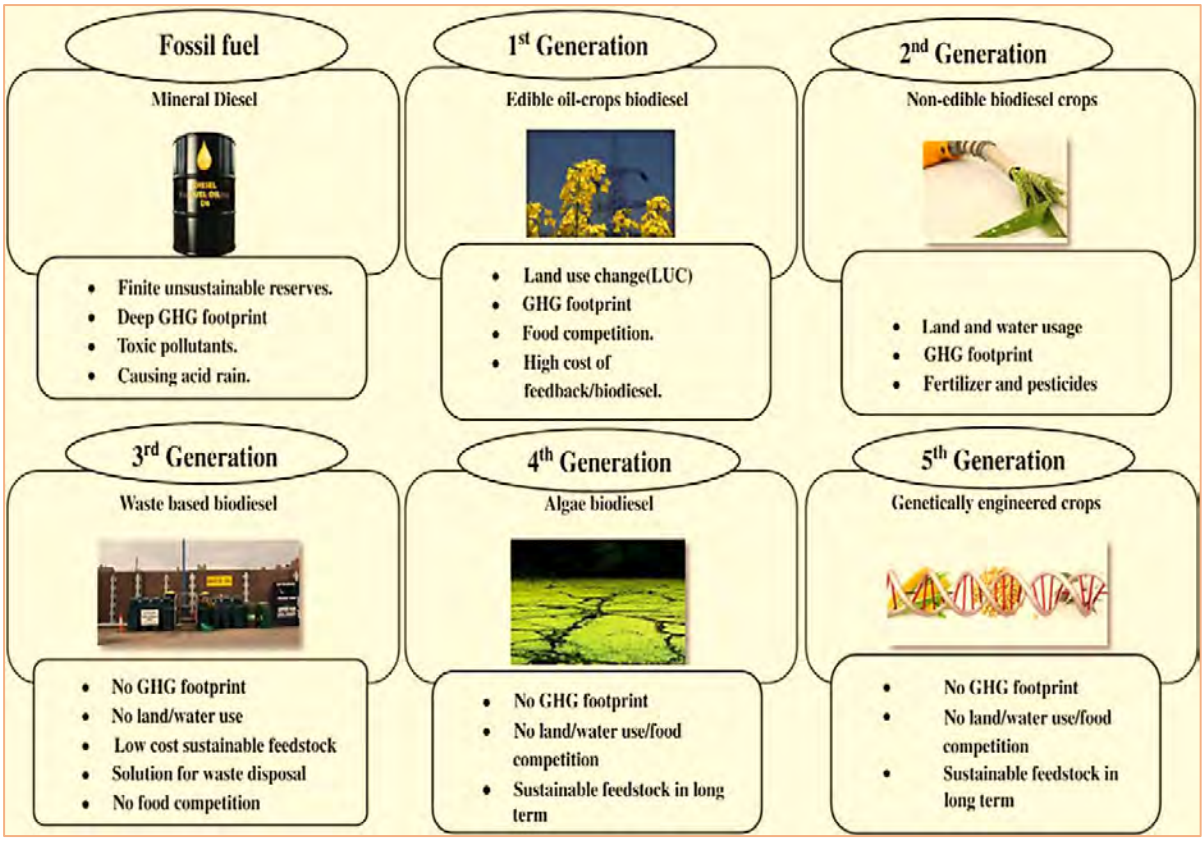


Fig. 2.3. Classification of feedstocks for biodiesel synthesis (ACS Omega 2021)

(Zulqarnain et al., 2021).

2.5.2.1. First and second-generation feed stocks for biofuels

The two basic categories for biomass are first and second-generation feedstocks. Edible food crops including sunflower, corn, maize, soybean, wheat, and rapeseed are among of the widely recognised first-generation feedstocks that can be utilised to make biofuels and commodities. Starch-based biogas, bioethanol, and biodiesel are the most utilised first-generation biofuels. Various feedstocks that contain fermentable sugars and carbohydrates are fermented to produce bioethanol. Sugarcane, sugar beetroot, and plants that produce starch, such maize and wheat, are used as feedstocks in the production of bioethanol. According to (Abud & Silva, 2019), there were 45–50 million tons of oil equivalent (MTOE) produced globally as bioethanol. The United States of America (USA) produces the most bioethanol (69.32 billion liters annually), primarily (about 95%) from corn and barely (about 3%) from other grains like sorghum, barley, or wheat starch. With an annual production of 27.25 billion liters of bioethanol from

sugarcane, Brazil ranks second in the world. The European Union (EU), based on sugar beetroot production, is the third-largest bioethanol producer in the world (Ballesteros and Manzanares, 2019). One of the most widely used biofuels is biodiesel, which is made from plants that are based on edible fats and oils such soybean, sunflower, rapeseed, and palm. About 31.6 billion liters of biodiesel were produced globally in 2015. According to (Naylor and Higgins, 2017), the EU, USA, and Brazil are the three economies that produce the most biodiesel, with annual production rates of 13.5, 4.8, and 4 billion liters, respectively. High sugar and fat content, minimal recalcitrance, and ease of processing into biofuels and related products are the most significant benefits of edible feedstocks for biorefinery purposes. Edible feedstocks have drawn attention from across the world, but their viability is hampered by issues including competition for the food supply and the need to acquire land for biofuels and other commodities, which drives up the price of food. Regarding its utilization, this competition raises ethical, political, and environmental issues. A different approach has been developed to make biofuels and related goods from the second generation of non-edible feedstocks, which are viewed as excellent choices for the development of biorefineries. Many lignocellulosic energy crops, herbaceous and woody biomass, forest and agricultural waste residues, municipal solid waste, and animal fats are examples of non-edible feedstock. Second generation feedstocks are plentiful and diverse in nature, and they can be processed using a variety of technologies to produce biofuels. In terms of land use, efficiency, and environmental performance, the biofuels and value-added goods produced from second-generation feedstocks offer some advantages over those produced from first-generation feedstocks (Searcy and Flynn, 2008) (Fleming et al., 2006).

The technologies that use second-generation feedstocks as feedstock are still in the early phases of commercialization, even though they are typically more widely accessible, more economical, and more widely used in the production of biofuels and value-added goods.

However, these technologies might advance quickly and will soon dominate the entire market (Kamm et al., 2005). Non-edible crops such as Mahua, Neem, Moringa,

Karanja, Polanga, Jatropha and castor are used as second-generation feedstocks to produce biofuels and commodities (Islam et al. 2018) (Chinnici et al. 2018).

The fact that most of these non-edible feedstocks are raised on agricultural land, however, inadvertently adds to the conflict between feed and fuel to some extent. In contrast, the castor plant in the setting of biorefineries presents several advantages over these other non-edible feedstocks.

The choice of feedstock is one of the most crucial factors to consider for biodiesel production. The feedstock covers almost 75% of the cost of manufacturing biodiesel. The amount of oil in the feedstock is determined by its kind alone. Figure 2.4 displays the breakdown for biodiesel production.

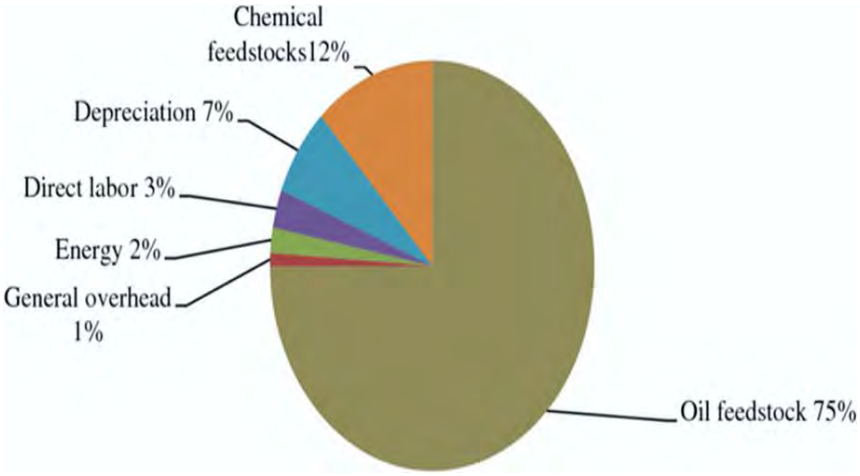


Fig. 2.4. Total cost breakdown for biodiesel production (ACS Omega 2021)

(Zulqarnain et al., 2021).

2.5.3. Properties of Biodiesel

Biodiesel is a yellow to dark brown liquid with a high boiling point up to 200^{O}C and low vapour pressure i.e., less than 1mm of Hg at 22^{o}C that is scarcely miscible with water, depending on the raw material used to make it. It has less sulphur than mineral diesel and doesn't have any benzene or other aromatics. The flash point is above 130°C and is therefore significantly higher than that of regular diesel, because of this property biodiesel is not a dangerous substance with a distillation range of 195-325^{o}C. Biodiesel typically has a stable reactivity, a mild musty and soapy odour, and is insoluble in water.

Additionally, studies claim that biofuels can cut GHG by up to 65%. These characteristics of biodiesel make it the ideal alternative fuel and allow for its widespread usage in many nations, particularly in ecologically sensitive areas (Goodrum, 2002).

2.5.4. Biodiesel as Vehicle fuel

Biodiesel is utilised as a substitute for Petro-diesel since it shares many characteristics with that fuel and may be utilized in diesel engines without requiring any changes. As a result, biodiesel as a fuel for cars increases: Energy security, the environment and air quality, and offers safety advantages.

2.6. Factors affecting transesterification reaction

The efficiency of the biodiesel process depends on a variety of variables, including the molar ratio of alcohol to oil, catalyst concentration, time for reaction, and agitation.

2.6.1. Molar ratio

Typically, primary and secondary alcohols are used in transesterification (Onukwuli et al., 2017). Methanol is commonly used alcohol because it is a cheap alcohol and exhibits chemical benefits due to its polar nature and short carbon chain (Balat & Balat, 2010) (Leung et al., 2010). Additionally, it does not produce zoetrope, making recovery easier than with ethanol (Musa, 2016) (Keera, Sabagh, et al., 2018).

To produce biodiesel, the molar ratio of triglycerides to alcohol is essential. Although in practise more alcohol is needed to advance the reaction and reduce the chance of alcohol evaporation, for biodiesel production ratio of triglycerides to alcohol is 1:3. According to past studies, the optimal molar ratio of oil to alcohol for the production of biodiesel was frequently determined to be 1:6. If the molar ratio of alcohol to oil is greater than the optimal ratio (1:6), the yield is not improved; rather, the process expenses are increased. Feed stocks which have high FFA content need higher oil to alcohol molar ratios up to 1:15, especially for acid catalysts (Yaakob et al., 2013).

2.6.2. Reaction time

Reaction time affects the rate of triglycerides conversion into esters. Fats and oils combine and spread alcohols; the reaction begins slowly. Later, the reaction rate rises until the optimal reaction time when it hits its peak. Reports state that the biodiesel

output peaks at reaction durations under 90 minutes and that adding more time after this point had no beneficial effect on the esters production (Alamu et al., 2007). Additionally, extending the reaction time beyond the optimal range reduces the product yield, which ultimately results in the loss of esters and the formation of FFA to make additional soap (Yaakob et al., 2013) (Eevera et al., 2009).

2.6.3. Reaction temperature

Reaction temperature has an impact on biodiesel synthesis as well. As temperature rises, it also affects oil's viscosity and response time, both decreases at high temperature. If the temperature of reaction is increased beyond its optimal range, soap will be produced. Alcohol will quickly evaporate at temperatures over its boiling point; hence this should not be done. The optimal temperature for producing biodiesel is normally between 50°C and 60°C, based on the type of oil utilised (Leung & Guo, 2006).

2.6.4. Catalyst concentration

Heterogeneous acids, homogeneous alkalis, or enzymes can all be used as catalysts in the transesterification process (Keera, Sabagh, et al., 2018). The esters yield can be increased in large part by raising the catalyst concentration. For the manufacture of biodiesel to be effective, more catalyst must be used. The development of soap will arise from increasing the catalyst past its optimal level, which will result in the loss of esters and greater process costs. According to several studies, the ideal catalyst concentration for a higher biodiesel output is 1.5% (Eevera et al., 2009) (Leung & Guo, 2006).

2.6.5. Agitation speed

Speed of agitation is crucial in the creation of the final product, as stirring of the oil and catalyst increases the reaction speed. The formation of the finished product (mono alkyl ester or biodiesel) depends on how quickly the oil and catalyst combination are stirred because doing so speeds up the process. For instance, the mixing intensities can be 300, 400, 600, and 900 rpm while all other parameters remain the same. Reduced product output can be seen by slower stirring. On the other hand, faster stirring enhances the formation of soap (Asif et al., 2017).

Ricinus communis L

2.7. Background

The castor plant is scientifically called *Ricinus communis L*. It is a member of the *Euphorbiaceae*. *Ricinus* basically originated from tick of European sheep that is Ixodes Ricinus or sheep tick and *communis* in Latin used for "common" because genus has only these species. In 1803 castor plant was introduced in Australia for the first time. *Ricinus communis* has many common and dialectal names in different countries. In Pakistan it is called as castor oil plant (Bueno et al., 2017).

2.8. Morphology, Habitat and Germination Period

North-eastern Africa and Eurasia have unique plants. It grows in tropical and subtropical dry areas as well as in different areas with hot summer (M. Das et al., 2018). In Pakistan it is found at Sub-Himalayan track and also in plain areas (Iqbal et al., 2012). It has an annual, biennial, or perennial life cycle and it grows fast. It has hollow, stiff, pale green and red branches. The old branches show a grey color. Its germination period includes autumn to spring season. The castor plant is a large, palmate, and lobed plant with deep lobes and brilliant green leaves. Its thick, hollow stems give it a treelike appearance, with violet or reddish hues and tiny hairs. The plant produces small, unisexual flowers, with female flowers at the bottom and male flowers at the top. After pollination, female flowers transform into seed pods or capsules, which release seeds as they ripen. The castor plant's deep root system allows it to thrive in various soil types and endure drought conditions. Seed production starts from six month and it continues 6-7 months yielding throughout the year (Yesilyurt et al., 2020). It shows slow growth in winter and sometimes plants die while in spring plants show rapid growth. Its output volume starts to decline after the third generation, at which point planting must be recommended. In frosty areas it behaves as annual crop (Gad et al., 2018). It can grow in land that is undeveloped and marginal (Sundus et al., 2017). Its cultivation cost is low, and it can sustain different weather conditions as well as produce higher oil yield (45–50%). Castor seed is used to extract oil, which is mostly made up of non-drying, ricinoleic acid triglycerides and accounts for over 95% of castor seed use.

2.9. Seed

Castor plant seeds, also referred to as castor beans, are oval-shaped, substantial, lustrous, and have a distinct mottled pattern. They typically have a length of 1 to 1.5 centimeters and 5 to12 mm width. Seed production starts from the first year. Castor seeds have a thick, hard bright brown red in color shell called hull that protects the white, oily endosperm inside, which is where castor oil is made. Hull is easily separated from kernel. The hull contributes 20 to 30% of seed weight (A. Das et al., 2019).

2.10. Oil

The oil from *R. communis L* is light or pale yellow in color and has a mild flavor or aroma. Seed oil has a high density or thickness 961kg/m^3, with a boiling point of about 313°C (Aziz et al., 2016). Castor seed typically contains 40-55% oil in comparison to the majority of other regularly used oil crops sunflower: 25-35% (w/w), rapeseed: 38-46% (w/w), soybean: 15-20% (w/w), and palm: 30-60% (w/w). Furthermore, the cost of cultivation can be equivalent to 25% of the price of jatropha and 50% of the cost of rapeseed.

Castor beans are not suitable for human consumption, thus using them as a source of energy does not interfere with growing food (Keera, Sabagh, et al., 2018). 80–90% of fatty acids in castor oil are hydroxylated, primarily ricinoleic acid, while 10% are non-hydroxylated fatty acids, primarily oleic (2.8 %) and linoleic acids (4.4%) and linolenic acid (0.2%) (Keera, Sabagh, et al., 2018) (Panhwar, et al., 2016) (Aziz et al., 2016) (Jena & Gupta, 2012).

Table 1: ASTM specifications for castor oil

Following are the some ASTM specifications for castor oil (Panhwar et al., 2016).

Factors	ASTM
Oil (%)	-
Specific gravity at 28˚C (g/cm^{-3})	0.957-0.968
Refractive Index at 28˚C	1.476-1.479
Viscosity at 28˚C (mPas.s)	630-880
Moisture Content (%)	0.001-2.5
FFA (%)	0.4-4.0
Iodine Value (gI_2/100g)	82-88
Peroxide Value (meq/Kg)	≤5
Saponification Value (mgKOH/g)	175-187

2.11. Castor's global production

Castor is cultivated in thirty countries on a commercial level for oil production which produces 220,000 tons per annum. Brazil, China, Russia, Thailand, and India produce castor oil in large quantity. India contribute 70% of the total export (Panhwar et al., 2016). Global production of castor oil climbed by 1.8 million tons annually in 2018 (Attia et al., 2018). Despite significant fluctuations in recent years, Pakistan's castor oil seed production generally decreased from 1972 to 2021, reaching a peak of 3,795 tons in 2021 (knoema, 2022).

2.12. Phytochemicals

Numerous substances, such as triterpenoids, saponins, resins, tannins, alkaloids, lignin, glycosides, and flavonoids, have been found in *Ricinus communis L*. Alkaloids are among the most significant of these substances. Its main components are found in the leaves, roots, and seeds (Aziz et al., 2016).

Fig. 2.5. *Ricinus communis L* oil, seeds, and leaves

(Chidambaranathan, et al., 2020) (Yeboah et al., 2020)

2.13. *Ricinus communis* Biorefinery

Medicinal and illumination are its traditional usage (García et al., 2017). These days, the chemical industries employ it as a versatile raw material for biodiesel production. It is used is used as an alternative diesel engine fuel (Arbab et al., 2013).

2.14. Castor seed oil-derived biodiesel characteristics

Castor oil is used to create biodiesel, which has several distinctive qualities that make it an appealing replacement for traditional fuels. Castor biodiesel offers several benefits as a renewable and sustainable energy source, as well as several unique qualities that affect its performance and use. Castor oil has a large molecular weight (298g/mol), a relatively low melting point (5°C), unsaturated bonds, and low freezing point (12 to 18°C), all of which contribute to its commercial value and application. Castor oil's kinematic viscosity lowers during transesterification, bringing it into line with other oils and making it appropriate for biodiesel mixes.

As a result, less energy is used during transesterification of castor oil. The flash point of biodiesel made from castor oil is 260°C, its iodine value is 82-88, the heating value is 39.5 GJ/ton, the sulfur and potassium contents are at a minimum, and the ash levels

are at 0.04 and 0.02%, respectively. The castor plant has a high potential for carbon trading and consumes 34.6 tons of CO_2/ha. In Pakistan, castor oil is a desirable option for the manufacturing of biodiesel (Zulqarnain et al., 2021).

The following are the primary attributes of castor oil-derived biodiesel:

2.14.1. High viscosity

Castor biodiesel is well-known for having a high viscosity, or, more specifically, for its resistance to flowing. Its particular composition results in a viscosity that is almost seven times greater than that of typical vegetable oils (Banković-Ilić et al., 2012). This characteristic has benefits and drawbacks. High viscosity improves biodiesel's lubricating qualities, making it a great ingredient for lubricants and hydraulic fluids. It necessitates careful consideration during the blending process because it can potentially result in problems with cold flow, particularly in colder locations. Castor oil biodiesel has attained effective specifications in standards when blended with petroleum-based diesel to overcome this drawback. Castor oil differs from other oils due to the hydroxyl group (OH) attached to the hydrocarbon chain in the ricinoleic acid molecule. It has a high viscosity and polarity that makes it particularly beneficial for the industrial manufacturing of coatings, polymers, and cosmetics. It also has a higher lubricity than other vegetable oils and works well as a diesel fuel additive.

The effects of increasing its solubility in alcohol include a lower melting point and improved oxidation stability. It has good cold flow properties since it contains a significant quantity of unsaturated fatty acids. Due to castor oil's incredibly high solubility in alcohol, it can be converted into biodiesel even at low temperatures (Keera, Sabagh, et al., 2018).

2.14.2. Ricinoleic acid content

The high concentration of the unique fatty acid ricinoleic acid in castor oil sets it apart from other vegetable oils. Castor biodiesel has desired qualities like better lubricity and a higher cetane number, which are a result of its high ricinoleic acid content. An engine's performance and combustion are improved with a greater cetane number.

2.14.3. Low Sulfur Content

Castor biodiesel's low sulfur content is one of the fuel's main benefits. Biodiesel contains a very little amount of sulfur in comparison to traditional diesel fuels, which lowers the dangerous sulfur dioxide emissions that cause poor air quality and less acid rain to occur (Keera, Sabagh, et al., 2018).

2.14.4. Biodegradability

Castor oil is used to make biodiesel, which means it can degrade harmlessly in the environment. Since it reduces the danger of pollution and contamination, biodegradability is a key component in lowering the total environmental impact of fuel consumption (Martínez et al., 2018).

2.14.5. Renewable Resource

Castor oil, the main ingredient in the manufacturing of castor biodiesel, comes from the castor plant, a renewable resource. Castor oil may be sustainably produced through agricultural practices, making it an environment friendly substitute for fossil fuels (Keera, Sabagh, et al., 2018) (Akhtar, 2023).

2.14.6. Diesel engine compatibility

Most diesel engines can run on castor biodiesel without any engine modifications. Due to this quality, it is an easy transfer for current diesel-powered machinery and vehicles, making it a feasible choice for wide adoption (Rao et al., 2017).

2.14.7. Reduced toxic emissions

Castor biodiesel has lower amounts of toxic emissions, including carbon monoxide and hydrocarbons, which improves air quality and is better for people's health (Demirbas, 2009) (Singh & Singh, 2010).

In conclusion, castor oil is a great source of biodiesel because of its high viscosity, low sulfur level, and biodegradability. It is a promising contender for a greener and more sustainable energy future due to its compatibility with current diesel engines and lower greenhouse gas emissions. Castor biodiesel's special qualities are likely to play an

increasingly important part in the worldwide drive to reduce reliance on fossil fuels and battle climate change as biodiesel technology.

Biodiesel increases the lubricity of fuel while also increasing the octane number of gasolines. A greater cetane number causes the engine to fire up more rapidly and with less delay. Diesel engines depend on the fuel's lubricity to stop moving parts from wearing out too quickly. Increasing the lubricity of the moving parts reduces friction, which prevents further wear. One of the main benefits of biodiesel is that it can increase the lubricity of the fuel even at blend levels as low as 1% (U.S Department of Energy).

2.15. Applications of castor oil biodiesel

- Castor oil biodiesel can be used in vehicles including cars, trucks, buses, and trains, in place of conventional diesel fuel. Depending on the climate and engine compatibility, it can be used as pure biodiesel (B100) or blended with Petro-diesel in a variety of ratios (Zulqarnain et al., 2021).
- Castor oil biodiesel can be used in stationary engines to produce power as well. It can be used in places that are off the grid or in power plants that need renewable energy sources.
- The use of biodiesel derived from castor oil in agricultural machinery and equipment can lessen the impact of farming activities on the environment and their reliance on fossil fuels.
- Several businesses need a regular and dependable source of energy to heat, process, or run industrial gear. In these situations, castor oil derived biodiesel can be utilized as a renewable substitute for standard fuels (Ogunkunle & Ahmed, 2019).
- Boats and ships can run on biodiesel generated from castor oil. It could lessen greenhouse gas emissions and the negative effects of maritime traffic on the environment.
- Though less prevalent than other uses, biodiesel has been tested as an aviation fuel in a few experimental aircraft. Biodiesel made from castor oil might be a possibility for these kinds of aviation tests.

- Castor oil has great lubricating qualities, and its derivatives have been employed in lubricants, hydraulic fluids, and additives in a variety of industrial applications (Estevez et al., 2022) (Vilas Bôas et al., 2022). Inks, dyes, plastics, hydraulic and brake fluids, soaps, lubricants, and hydraulic and brake fluids are just a few of the industrial uses for castor oil. It is highly prized for its lubricating qualities and capacity to retain viscosity at very high temperatures (Onukwuli et al., 2017).

2.16. Antibiotic resistance

Antimicrobial resistance (AMR) is the phenomenon whereby microbes develop over time and lose the ability to be treated by antimicrobial medications that once worked against them. Infections spread and become more challenging to cure because of this treatment resistance.

Alexander Fleming discovered penicillin in 1928. The world was inspired to discover more antimicrobial medicines to prevent and treat serious diseases as the era of antibiotics began with this groundbreaking discovery. Only fifty years later prevention and treatment became successful. Numerous lives have been saved by antimicrobial compounds, and the ratio of morbidity to mortality has decreased significantly. In human history, it marked a turning point. Sadly, the emergence of resisting microbes to antimicrobial compounds has reduced their usage (Davies and Davies, 2010).

2.16.1. Causes of Antibiotic resistance

The leading causes of antimicrobial resistance are bacterial penicillinase (McArthur et al., 2013), biofilm formation, efflux pumps (Høiby et al., 2010), horizontal gene transfer, transposon transfer (David M. Livermore,2000) (Bengtsson-Palme et al., 2018), (Davies and Davies, 2010), excessive use of antibiotics (McGowan, 1983), (Davies and Davies, 2010) and biochemical and physical mechanisms of microbes (Davies and Davies, 2010) (Martinez, 2014).

The most known microbes that have developed resistance are *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Serratia, Escherichia coli, Acinetobacter baumannii, Salmonella enteric, Staphylococcus aureus, Clostridium difficile* (Davies and Davies,

2010) (McGowan, 1983) *Candida albicans, Aspergillus species, Aspergillus fumigatus,* and *Fusarium* species (Wiederhold, 2017).

2.16.2. Plant-based antimicrobial drugs

Antibiotic resistance has caused a global focus on developing plant-based antimicrobial drugs (Ameya et al., 2017) (Saeed and Tariq, 2005) (Pramila et al., 2012). 80% of people worldwide use plant extracts for therapeutic purposes as their primary source of healthcare (McKay and Blumberg, 2006) (Mahendran and Rahman, 2020). In various nations around the world, the antibacterial and antifungal activities of medicinal plants have been addressed.

According to WHO, in 80% of traditional medicines, plant extracts and their active components are employed. Secondary metabolites found in plant leaves, seeds, oil, and stem have antimicrobial effects on a wide range of microbes (Anumudu et al., 2019) (Al-Lahham et al., 2020), (Pramila et al., 2012) (Capdesuñer et al., 2019).

Many ailments can be treated with plants and various herbs, and they are also offering alternatives to conventional drugs and therapies (Pramila et al., 2012). About 50,000 plant species can produce different compounds (Al-Lahham et al., 2020) (Andoan et al., 2002). Plants have produced 500,000 bioactive metabolites, and these metabolites have been effectively exploited to create pharmaceutical medications (Ameya et al., 2017).

Synthetically manufactured chemicals are expensive, susceptible to resistance, and have side effects while plant-derived medicines are cheap, less susceptible to the development of resistance, and don’t have side effects (Ameya et al., 2017) (Anumudu et al., 2019) (Patil et al., 2015) (Pramila et al., 2012) (Saeed and Tariq, 2005) (Raghavan et al., 2018).

To create therapeutic reagents, the secondary plant metabolites, or phytochemicals, have undergone extensive study. The existence of secondary plant metabolites with antibacterial and antifungal properties is so predicated. Different plant components and metabolites have been utilized to cure and prevent various ailments since ancient times (Anumudu et al., 2019).

2.17. Phyto-Pharmacology applications of *Ricinus communis L*

2.17.1. Castor oil usage

2.17.1.1. Medicinal usage

For its many health advantages, castor has a long history of usage in traditional medicine. Due to its ability to encourage bowel movements and treat constipation, it is frequently used as a laxative. In addition to its usage as a laxative, castor oil is occasionally a component of pharmaceutical products such as ointments, and eye drops. Castor seed oil is a useful and adaptable natural substance that has a wide range of uses in industry, medicine, and cosmetics. It's critical to seek medical advice before using any natural remedies.

2.17.1.2. Cosmetics and skincare products

Castor oil is frequently used in cosmetic and skincare products because of its nourishing and moisturizing qualities. It can be used topically on the skin to relieve dryness, lessen inflammation, and speed up the healing of wounds. Castor oil is thought to strengthen hair, lessen breakage, and promote hair growth when used in hair care (V. R. Patel et al., 2016) (Jena & Gupta, 2012).

2.17.2. Medicinal properties of Castor seeds and extracts

2.17.2.1. Antimicrobial activity

Castor shows good antimicrobial activities against dermatophytes and pathogenic bacterial and fungal strains e.g. *Streptococcus progenies*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Bacillus subtilis*, *Aspergillus flavus*, *Candida albicans*, *Curvularia lunata*, *Fusarium oxysporum* and *Aspergillus niger* (Verma et al., 2011) (Jena & Gupta, 2012) (Soni & Dhiman, 2017).

2.17.2.2. Antioxidant activity

Castor extracts have antioxidant and free radical scavenging properties. *R. communis L* seed exhibits strong antioxidant activity even at low concentrations, suggesting that it may be particularly effective in treating oxidative stress associated diseases. Methyl

ricinoleate, Ricinoleic acid, 12 octadecadienoic acid, and methyl ester are the chemical components responsible for antioxidant action. Stem and leaf extracts also induce antioxidant activity since flavonoids are present in these extracts (Jena & Gupta, 2012) (Bhaumik et al., 2018). Previous study (Rana et al., 2016) showed that major antioxidant properties of *R. communis L* are due to phenolic compounds (gallic acid, gentisic acid, ellagic acid, epicatechin, quercetin, and rutin).

2.17.2.3. Antinociceptive activity

Castor has significant antinociceptive activity. Preliminary phytoconstituents such as saponins, steroids, and alkaloids were present, which caused the antinociceptive activity to manifest (Jena & Gupta, 2012) (Taur et al., 2011).

2.17.2.4. Antiasthmatic and Antihistaminic activity

Castor seed extracts possess antiasthmatic and antihistaminic activities. As a result of its ability for stabilizing mast cells and providing anti-allergic benefits, *R. communis L* is useful in treating asthma. The flavonoids and saponins both have a stabilizing impact on mast cells apigenin and luteolin. Similarly, flavonoids can typically suppress basophil, histamine, and neutrophil beta-glucuronidase release, and eventually demonstrates in-vivo antiallergic properties. They also have smooth muscle relaxant and bronchodilator effect. *R. communis L* has an antiasthmatic effect and reduces leucocytosis and eosinophilia caused by milk due to the inclusion of flavonoids or saponins (Jena & Gupta, 2012).

2.17.2.5. Anti- fertility activity

The *R. communis L* seed has steroids and alkaloids. Sex hormones cause the pituitary gland to release gonadotrophins. Combined effects of both estrogen and progesterone through both positive and negative feedback mechanisms, the pituitary gland inhibits the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) during the luteal phase of the menstrual cycle. The follicle's ability to mature in the ovary is inhibited, which helps to avoid ovulation. Anti-fertility effects are brought on by the steroidal properties of sex hormones (phytosterols) and the presence of steroids in *Ricinus communis* seed extract (Jena & Gupta, 2012) (Sandhyakumary et al., 2003) (Khan Marwat et al., 2017).

2.17.2.6. Immune system modulatory activity

Immunomodulatory substances with both plant and animal origins typically boost the body's ability to respond to infections by engaging the non-specific immune system. Leucocytes ingest microorganisms during phagocytosis. In last, neutrophils' ability to kill bacteria inside their cells is known as phagocytosis. Human neutrophils' phagocytic activity is greatly boosted by the tannins in *R. communis*, and this may have produced an immune-modulating effect 22 (Jena & Gupta, 2012) (Moraes et al., 2019).

2.17.2.7. Hepatoprotective activity

Castor exhibits hepatoprotective properties. The liver's lipid peroxidation, protein, glycogen, and acid and alkaline phosphatase activities are all inhibited by *R. communis* extracts, which also reduces the activity of serum transaminases. Glutathione levels and adenosine triphosphatase activity depletion can both be treated with the *R. communis L* extract. Flavonoids present in *R. communis L* have positive effects. The antioxidant and membrane-stabilizing properties of flavonoids are important. The presence of flavonoids and tannins in *R. communis L* increases the liver's potential for regeneration and repair. *Ricinus communis Linn* shows anti-cholestatic and hepatoprotective effects due to N-dimethyl ricinine against paracetamol-induced liver injury. *Ricinus communis* also has a great effect against liver necrosis and fatty alteration (Jena & Gupta, 2012) (D. Ray et al., 2006) (Kumar, 2017).

2.17.2.8. Anti-inflammatory activity

R. communis L has great potential in preventing cellular events during the development of edema as well as in all phases of acute inflammation. The presence of flavonoids in *R. communis L* is responsible for its anti-inflammatory properties (A. K. Saini et al., 2010) (Jena & Gupta, 2012) (Mesaik et al., 2018).

2.17.2.9. Wound healing activity

The tannins, triterpenoids, flavonoids, and sesquiterpenes have astringent and antibacterial effects, encourage the wound-healing process. They cause wound contraction and a fast rate of epithelialization. Castor oil's active components produce antioxidant properties and inhibit lipid peroxidation to help in the healing of wounds.

It is believed that chemicals that prevent lipid peroxidation increase collagen fiber strength, increase circulation, lessen cell damage, and enhance DNA synthesis, all of which contribute to the viability of collagen fibrils (Jena & Gupta, 2012) (S. Patel et al., 2021) (Díez-Pascual & Díez-Vicente, 2015).

Castor has great potential as a substrate for biorefinery. Its seeds have the potential for biodiesel production as well as to reduce the dependence on antimicrobial compounds. In the current study, after mechanical extraction of seed oil, seed-compressed seed cake was utilized for the preparation of extracts in different solvents like methanol, chloroform, n-Hexane, methyl acetate, and ethyl acetate. The antimicrobial potential of both oil and extracts was evaluated by the agar well diffusion method against bacterial (ATCC and MDR) and fungal strains. MICs were evaluated by the microtiter plate method. Different chemical tests and FTIR were used to screen the phytochemical content of extracts and oil. Extracted oil was utilized to produce biodiesel as a renewable source of energy. Stat Ease design expert software version 7 was used to optimize the biodiesel production. Five factors were optimized, oil to methanol ratio, temperature, catalyst concentration, agitation, and reaction time. Placket Burman design was run to obtain the possible combinations of all the factors to achieve maximum biodiesel yield.

The *R. communis L* seed has been handled in the setting of a biorefinery in the current study, as shown in Figure 2.6.

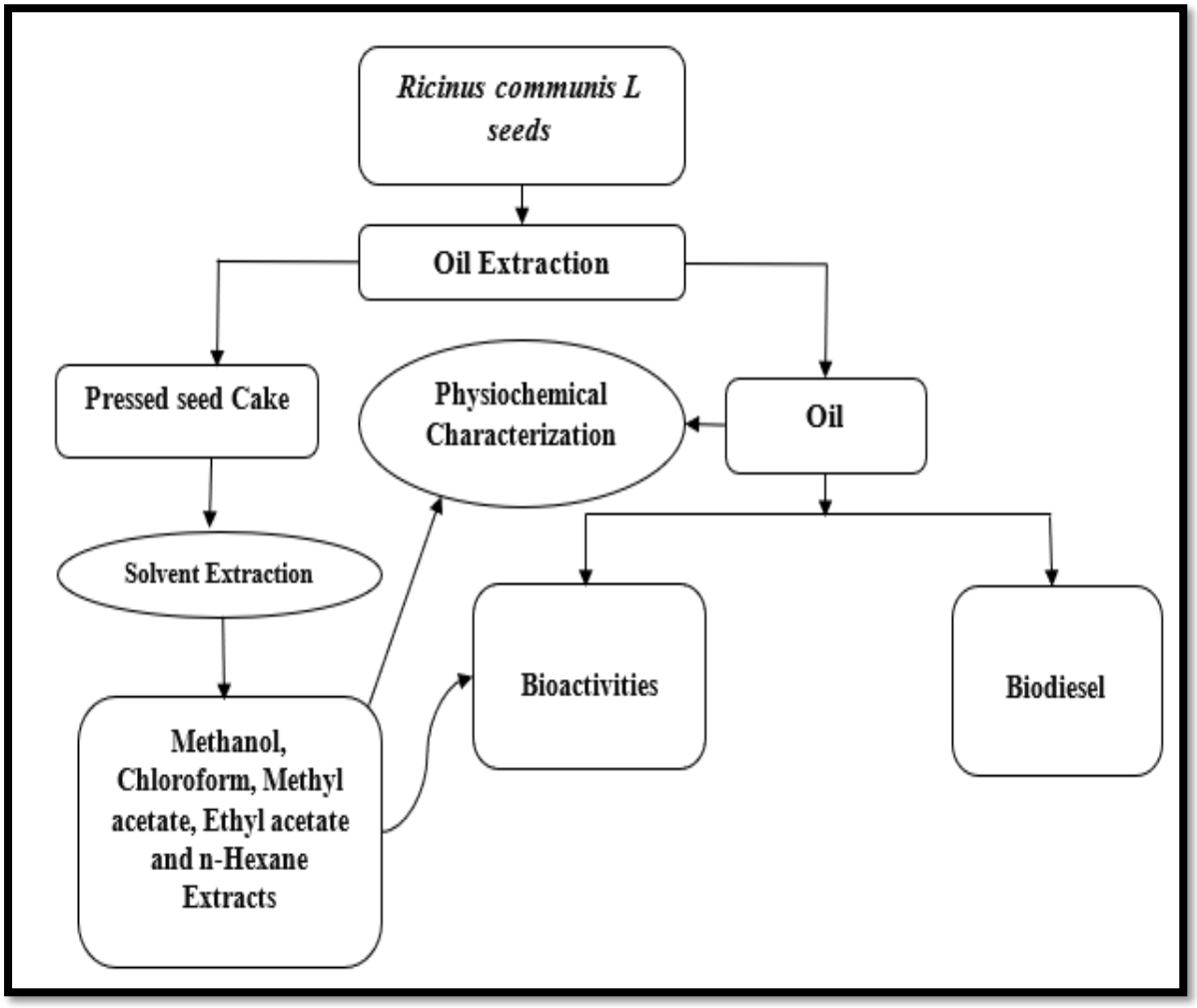


Fig. 2.6. Research plan for using *Ricinus communis L* seeds in a biorefinery setting.

Chapter # 3

Methodology

Methodology

The aim of the research was the detection of antimicrobial and biodiesel production potential from seeds *Ricinus communis L* plant. All the research work was performed in the Sustainable Bioenergy and Biorefinery Lab, Department of Microbiology, Quaid-I-Azam university Islamabad, Pakistan.

3.1. *Ricinus communis L* seed Oil Extraction

Ricinus communis L seeds were obtained from a family friend who belongs to Mianwali, Pakistan, and identified by Herbarium of Pakistan. Oil was mechanically extracted from the seeds with the help of an expeller. After oil extraction seed cake was air dried to evaporate moisture content. It was further ground to obtain the fine powder after that stored in sterile zipper bags for further use. The extracted oil was filtered through Whatman filter paper for the removal of contaminants and after that heated at 105°C for a few hours to remove the moisture content, then stored in the dark for later use. The de-oiled seed cake was kept at 4°C in sterile zipper bags after the oil extraction.

3.2. Oil Yield

The following formula was used to determine the oil yield.

Oil yield (%) = (Oil extracted in Liters / Total weight of seeds in kg) × 100

3.3. Extract Preparation

Extracts were prepared from seed cake as described in (Akpan et al., 2006). 100g of seed cake fine powdered was added in 500ml of solvents separately and placed in shaking incubator for 12 days at 37°C at 100rpm. Methanol, methyl acetate, ethyl acetate, n-Hexane, and chloroform were used as solvents in the experiment. After removing from the shaking incubator solutions were filtered through filter paper. Filtrates were placed at room temperature to concentrate for one week. The yield of solutions of Methanol, methyl acetate, ethyl acetate, n-Hexane, and chloroform were calculated. Each extract (500mg) was dissolved in 1ml of DMSO for later use. Tetracycline is a broad-range antibiotic. It was used as a positive control. DMSO doesn't have antimicrobial activity and it was used as negative control.

3.3.1. Sterility Test for extracts

A sterility test was performed to test the sterility of the extracts. Extracts were filtered through sterile syringe filters of pour size 0.45µm. 100µl of each extract was spread on MHA. Then to confirm sterility incubated for 24 hours at 37°C (I. Haq et al., 2012).

3.4. Phytochemical screening

Using qualitative phytochemical analysis, the extracts, and seed oil from *Ricinus communis L* were screened for phytochemical content.

3.4.1. Qualitative phytochemical screening

By using the procedures outlined below, phytochemicals such as steroids, alkaloids, saponins, flavonoids, glycosides, resins/balsams, tannins, and phenols were qualitatively tested in *Ricinus communis L* seed oil and extracts.

3.4.1.1. Alkaloids

3.4.1.1.1. Mayer's Test

Two milliliters (2ml) of 10% aqueous HCl was added to 2ml of extract and oil and stirred. A few drops of Mayer's reagent were added to one milliliter (1ml) of the filtrate.

3.4.1.1.2. Wagner's Test

Wagner's reagent was used to treat one milliliter of the filtrate with a few drops of the reagent.

In both tests, the emergence of a creamy precipitate demonstrated the presence of alkaloids in the extract and oil. Alkaloids were also detected in the extract by a reddish-brown precipitate.

3.4.1.2. Flavonoids

Using sodium hydroxide, a three-milliliter (3ml) portion of the filtrate was turned alkaline. The appearance of a yellow color suggested the potential existence of flavonoid molecules**.**

3.4.1.3 Tannins and Phenols

2-3 ml of the extract were slowly added to a 5% ferric chloride solution. The existence of tannins is indicated by a precipitate that is dark green.

3.4.1.4. Resins

90 percent ethanol was combined with two milliliters (2ml) of each extract and oil. The combination was given 2 drops of an alcoholic ferric chloride solution. A dark green tint was seen.

3.4.1.5. Glycosides

2.5 ml of each extract, and oil was mixed with 5 ml of 50% H_2SO4 and boiled for 15 minutes in boiling water to detect the presence of glycosides. We used 10% NaOH solution to neutralize the mixture after allowing it to cool. Fehling's solutions A and B mixed in a 10 mL volume (1:1) were then heated again for 5 minutes. The strong brick-red precipitate development indicated glycoside presence.

3.4.1.6. Steroids

Each extract and oil were mixed in 5ml of chloroform using 5 grams (5g) of the material. To create the lower layer, two milliliters of concentrated sulfuric acid were carefully applied. The presence of steroids is indicated by the reddish-brown ring-colored steroidal ring at the interface.

3.4.1.7. Saponins

2.5 ml of Fehling solution A and B was added in 2.5 ml of extract. Bluish green ppts indicate the presence of saponins.

3.5. FTIR (Fourier transform infrared analysis)

400-4000 cm^{-1} scan range of Fourier transform infrared (FTIR) spectroscopy (Bruker Tensor 27) was used to investigate *R. communis L* extracts and seed oil.

3.6. Bacterial Culture Maintenance

Human pathogenic ATCC and MDR bacterial strains were selected. Select strains included Gram positive (*Bacillus subtilis, Staphylococcus epidermidis, Staphylococcus aureus*) and Gram negative (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella enterica, Enterobacter*) and MDR strains (*Salmonella, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). Every strain was cultured, maintained, and sub-cultured on fresh nutrient agar media at regular intervals and kept at 4°C.

3.7. Bacterial Suspension Preparation

250 ml nutrient broth was prepared and autoclaved. The media was poured equally into 11 flasks of 30ml. The media was inoculated with 11 selected bacterial strains and was placed for 24 hours in a shaking incubator at 37^{o}C. After the incubation, the cultured media was poured into falcon tubes and weighted. After equalizing the weight of each falcon tube, they were centrifuged at 4000rpm for 5 minutes at 4^{o}C. The centrifugation caused the formation of supernatant and pallets at the bottom of the tubes. The supernatant was discarded, and 20ml of normal saline solution was added to each tube. Again, weighed and centrifuged for 5 minutes at 4^{o}C and 4000 rpm. The supernatant was again discarded, and 20ml normal saline solution was added to each tube. The suspension was stored safely at 4^{o}C.

3.8. Antibacterial Activities

Antibacterial activities of. *R. communis L* seed oil and extracts were assessed by agar well diffusion method against ATCC and MDR strains. The standardized inocula was swabbed onto Mueller Hinton agar (MHA) growth media-containing plates by using sterile cotton swabs. The plates' solidified growing media was bored into 8mm diameter with sterile blue tips. 100µl of *R. communis L* seed oil and extracts each was poured into each well after it had been correctly labeled. Tetracycline was used as a positive control. DMSO served as the negative control. The inoculated petri plates were kept at room temperature for an hour before the bacterial growth to allow for treatment diffusion. The plates underwent a 24-hour incubation period at 37°C. ZOI were assessed following an incubation time (Kebede & Shibeshi, 2022).

3.9. MIC (Minimum Inhibitory Concentration)

Microtiter plates were used to estimate MIC of all treatments (*R. communis L* seed's oil and extracts) for ATCC and MDR strains. The treatments were diluted up to 10^{-4}, dilutions of the seed oil and extracts were prepared. 100µL of each target bacterial strain was inoculated into a well of a microtiter plate, and then 100µL of each treatment was added to the appropriate well. 100µl of each strain was used as control. The plates were incubated at 37°C for 24 hours. The MIC for each treatment was determined by spectroscopy at 600nm wavelength. The solutions' sterility was preserved throughout the experiment.

$$\mathbf{MIC\ (\%) = 100\ [C_{ab} - T_{ab}/\ C_{ab}]}$$

- ab = Absorbance
- C = Control
- T= Test sample

A value was determined to be the MIC when the turbidity of the extract or seed oil was reduced by 80% in contrast to the negative control. To confirm MICs, 20 µL of the extracts or seed oil from the wells with 80% reduction in growth was spread on MHA plates and incubated at 37°C for 24 hours (A. Haq et al., 2021).

3.10. Antifungal Activities

The human opportunistic pathogenic fungal strains (*Candida albicans, Aspergillus flavus, Aspergillus niger, Fusarium oxysporum,* and *Curvularia lunata*) and phytopathogenic strains (*Aspergillus flavus, Fusarium* and *Penicillium chrysogenum*) were selected. Fungal suspensions were prepared in normal saline. All the strains were cultured on SDA at 30°C for 48 hrs. The antifungal activities of extracts and oil were performed by the agar well diffusion method. The standardized inocula was swabbed onto the corresponding plates containing Sabouraud dextrose agar (SBA) growth medium using sterile cotton swabs. The hardened growth medium in the plates was drilled into an 8mm diameter well with sterile blues tips. After labeling each well, 100µl of *R. communis L* seed oil and extracts were put into it. Nilstat served as a positive control. The negative control was DMSO. To allow for treatment diffusion, the inoculation petri plates were placed at room temperature for one hour before incubation.

ZOI were measured after the plates had been incubated at 30°C for 48 hours (Kebede & Shibeshi, 2022).

3.11. MIC for fungal strains

MICs were determined by microtiter plates (96 wells). Up to 10^{-4} dilutions were prepared. 100µl of each dilution (ethyl acetate, methyl acetate, n-Hexane, chloroform, and methanol) of *R. communis L* seed oil and extracts were placed in separate wells together with 100µl of each fungal strain's suspension (in normal saline) and incubated for 48 hours at 30°C. Nilstat was utilized as a positive control and as a negative control, DMSO was utilized. The 100µl of each strain was used as control. MICs absorbance values were taken at 600nm. A value was determined to be the MIC when the turbidity of the extract or seed oil was reduced by 80% in contrast to the negative control. For each strain the percent growth was calculated by the following formula:

$$\mathbf{MIC\ (\%) = 100\ [C_{ab} - T_{ab}/\ C_{ab}]}$$

- ab = Absorbance
- C = Control
- T= Test sample

MHA plates were spread with 20µL of the extracts or seed oil from the wells with 80% reduction in growth, and the plates were then incubated at 30°C for 48 hours to confirm the MICs (A. Haq et al., 2021).

3.12. Antioxidant activity

3.12.1. 2, 2 diphenyl 1-picrylhydrazyl (DPPH) free scavenging assay

The ability of *R. communis L* oil and extract to scavenge free radicals against DPPH was tested according to (Iqbal et al., 2012). Dilutions of extracts, oil, and ascorbic acid were prepared with final concentration 0.05-500mg/ml. 5 dilutions of DMSO were also prepared (dilutions were prepared in distilled water). 20µl of each dilution of ascorbic acid, extract and oil was combined with 180µl of DPPH methanolic solution. Ascorbic acid was used as positive control while DMSO was used as negative control. Microtiter plate was incubated at room temperature in the dark for 30 minutes. After incubation, the sample's absorbance was taken at 517nm using a microplate reader. The shift in

color from purple to yellow also verified the antioxidant action. The following equation was used to compute the percentage of radical scavenging.

$$\textbf{DPPH Radical scavenging (\%) = 100 } [C_{ab} - T_{ab} / C_{ab}]$$

- ab = Absorbance
- C = Control
- T= Test sample

After calculating the percent antioxidant activities of extracts and oil, values of corresponding dilutions of DMSO were subtracted from their values and hence it gave original antioxidant % values of extract and oil (A. Haq et al., 2021).

3.13. Cytotoxic assay on brine shrimps

R. communis L oil and extracts' cytotoxic effects on brine shrimps (Artemia salina) were assessed. The artificial sea water solution was prepared by adding 38 g of artificial sea salt in 1 L of distilled water, filtered it through Whatman paper, and sterilized it in an autoclave at 121 °C and 15 psi pressure for 20 minutes. In sterilized artificial sea water near a light source at 37°C for 24 hours, about 1g of Artemia salina cysts (eggs) hatched. In a nutshell, 100µl of each prepared dilution of *R. communis L* oil and extract was poured into test vials containing separately 10ml artificial sea salt water. 10 active nauplii were added in each test vial. DMSO was utilized as a negative control, and physical control (sea salt and brine shrimps) was run to find out the effect of weather on brine shrimps. Vincristine sulfate was used as positive control. Dead and alive shrimps were counted after a 24 hour, and 48 hours incubation period (I. Haq et al., 2012) (A. Haq et al., 2021).

3.14. Oil's and biodiesel's physical-chemical characteristics

3.14.1. Acid Value and Percent free fatty acid (%FFA)

Acid value (AV) is the unit of measurement for potassium hydroxide required to neutralize the free fatty acids present in 1g of the substance (oil), expressed in milligrams (mg). It frequently tracks the conversion of free fatty acids from triacylglycerol, which is detrimental to the quality of many lipids and oils.

Materials

Oil, 0.1 N KOH, Absolute ethanol, Phenolphthalein, Erlenmeyer flask or Beaker.

Procedure

- 5.0 g of *R. communis L* oil is poured into dried 50 ml flask.
- Then 25ml of absolute ethanol and phenolphthalein's 2-3 drops were added.
- The mixture was warmed to 65ºC in a control temperature water bath with gentle shaking.
- The mixture was then cooled down and titrated against 0.1 N KOH solution until permanent pink color appeared, that is its endpoint.
- The amount of KOH solution was calculated.

The acid value (AV) and free fatty acid (%FFA) were calculated as:

AV= (ml of KOH) x N x 56 / Weight of sample= mg of KOH

% Free Fatty Acid (%FFA) = AV x 0.503

N = KOH Normality

3.14.2. Saponification value

The "saponification value is the amount of potassium hydroxide required to saponify the esters and neutralize the free acids in 1g of oil." It is a measurement of the triacylglycerols' average molecular weight in a sample.

Materials

Oil, 0.5 N Hydrochloric acid, Phenolphthalein, 0.5 N alcoholic potassium hydroxide, flasks.

Procedure

To make alcoholic KOH, 15g of potassium hydroxide was dissolved in 10ml water, and the final amount was increased to 500ml using 95% ethanol. For 24 hours, the solution was stored, followed by filtering through paper.

- Weighted 2 g of *R. communis L* oil was placed in a 100ml flask along with 25ml of a 0.5 N alcoholic potassium hydroxide solution.

- After that, the flask was connected to the reflux condenser and left in a water bath at control temperature for an hour while being sometimes shaken.
- Phenolphthalein indicator's 3 drops were poured into the solution after the first hour while it was still hot, and once the indicator had been added, the solution was titrated against 0.5 N hydrochloric acid until a permanent color disappeared, which is the endpoint.
- The Blank underwent the same process but without the inclusion of the oil sample and X ml of the 0.5 N HCL at the conclusion, denoted by B.
- The saponification value was calculated as:

$$\textbf{SP NO.} = \frac{\textbf{56.1 (B-S) x N of HCL}}{\textbf{Gram of sample}}$$

B: HCL (ml) needed by Blank

S: HCL (ml) needed by Sample

3.14.3. Ester value and % glycerin

The ester value is the quantity, measured in milligrams of potassium hydroxide, needed to react with esters present in one gram of oil or fat. Using a formula, it was determined from the acid value (AV) and the saponification value (SV).

$$\textbf{EV} = \textbf{SV} - \textbf{AV}$$

$$\textbf{\% Glycerin} = \textbf{EV x 0.054664}$$

3.15. Biodiesel Production

3.15.1. Alkali or Base Catalyzed Trans- esterification of *R. communis L* seed oil

Oil was poured into a flask. The oil was first preheated on a hot plate to become completely homogenized, and then 1% potassium hydroxide (based on the weight of the oil) was added to the methanol. After homogenization, a preheated chemical mixture of KOH and methanol was poured into oil containing flask which was then placed in a water bath. Temperature was adjusted with a temperature controller and constant stirring was done with a magnetic stirrer. The oil to methanol molar ratio was set to 1:6, the reaction temperature to 60^{o}C, the stirring at 600rpm, and the reaction time

to 2 hours. The reaction mixture was poured into a separating funnel after a two-hour reaction was completed, and it was given 24 hours to separate into layers.

3.15.2. Purification of Biodiesel

3.15.2.1. Methanol separation

Excess methanol must be removed from the biodiesel sample to purify the produced biodiesel. To achieve this, biodiesel was added to the distillation apparatus's round bottom flask, which had its temperature set to 65°C. After the distillation tube's opposite side flask's methanol collecting was stopped, the distillation equipment was halted. A quantity of pure biodiesel was recorded; it was kept in glass vials with screw caps and in the dark.

3.15.2.2. Alkali removal

By neutralizing and washing with acidified distilled water, KOH was removed. For this, 100ml of hot distilled water was mixed with 1ml of concentrated H_2SO_4. The trans-esterified mixture was then added to the acidified distilled water, which was then centrifuged for ten minutes at 3000rpm to separate the K_2SO_4 that had been produced. This process was repeated twice to ensure that no catalyst was still present. To remove water from the finished product, the catalyst-free product was once more centrifuged at 3000rpm for ten minutes. To remove any remaining moisture, anhydrous Na_2SO_4 was applied to the water-free product. There was a noticeable number of FAMEs. The following equation was used to determine FAME yield:

FAME yield (%) = (Weight of FAME (g) / Total weight of oil (g) × 100

3.16. Optimization of Reaction Parameters for Optimized Biodiesel Production using Plackett-Burman Design

Stat-Ease Design Expert Software version 7.0 was used to optimize response parameters using the Plackett-Burman design. Five factors were chosen for optimization and placed into design software: catalyst concentration, temperature, agitation, oil to methanol ratio, and reaction time. The design then produced 15 runs.

Table 3.1. Plackett-Burman Software Experimental design table

Runs	Catalyst Concentration (%)	Temperature (C)	Oil to Methanol ratio	Agitation (RPM)	Reaction time (min)
1	1.50	60.00	1:9	900.00	60.00
2	0.50	60.00	1:9	300.00	120.00
3	0.50	60.00	1:15	900.00	60.00
4	0.50	60.00	1:15	300.00	120.00
5	1.50	60.00	1:15	300.00	60.00
6	0.50	50.00	1:9	300.00	60.00
7	1.50	50.00	1:15	300.00	60.00
8	1:00	55.00	1:12	600.00	90.00
9	1:00	50	1:12	300.00	90.00
10	1:00	60	1:15	900.00	120.00
11	0.50	50.00	1:15	900.00	120.00
12	1.50	50.00	1:15	900.00	120.00
13	1.50	60.00	1:9	900.00	120.00
14	1.50	50.00	1:9	300.00	120.00
15	0.50	50.00	1:9	900.00	60.00

3.17. Biodiesel or FAME Analysis

Fatty acid methyl ester formed were analyzed by FTIR.

3.17.1. Fourier Transform Infrared Spectrometer

The methyl esters produced were identified and examined by FTIR utilizing a Bruker Tensor27 FTIR spectrophotometer and Opus65 software equipped ZnSe ATR. *R. communis L* oil and biodiesel samples totaling 5 microliters each were fed into sample injectors, where scans were carried out between 400 and 4000 cm^{-1}. The spectrum demonstrated various peak ranges.

Chapter # 4

Results

Results

4.1. Oil Yield

Through mechanical extraction, 20% oil was obtained.

Fig. 4.1. *R. communis L* seeds and extracted oil

4.2. Extracts yield

After the extract preparation, its yield was calculated and listed in Table 4.1.

Table 4.1. Extracts and their respective yields

Extracts	**Yield (%)**
Chloroform	4.35
Ethyl acetate	19.6
Methyl acetate	5.1
Methanol	10.15
n-Hexane	2.95

Fig. 4.2. Prepared extracts

4.3. Phytochemical analysis of *R. communis L* extracts and oil

To interpret the chemical composition of all extracts and oil of castor, several conventional experiments were performed. Qualitative tests were carried out to elucidate the major phytochemical components of the castor oil and pressed cake extracts. This conventional phytochemical confirmed the presence of several phytochemicals such as alkaloids, tannins/phenol flavonoids, steroids, glycosides, resins, and saponins in castor oil and pressed seed cake. Several phytochemicals pressed in seed cake and oil are listed in Table 4.2.

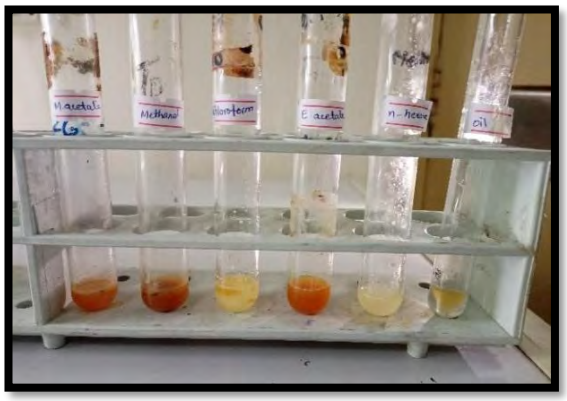
Table 4.2. Phytochemicals analysis of different extracts and oil

Extracts and oil	Alkaloids		Resins	Flavonoid	Glycosides	Steroids	Saponin	Tannins/ Phenol
	Mayer's test	Wagner's test						
Chloroform	+	+	-	+	+	+	+	-
Ethyl acetate	-	-	+	+	+	+	-	+
Methyl acetate	+	+	+	-	+	-	-	+
Methanol	-	-	+	-	+	-	+	+
n-Hexane	+	+	-	+	-	+	+	-
Oil	-	-	-	+	-	+	-	-

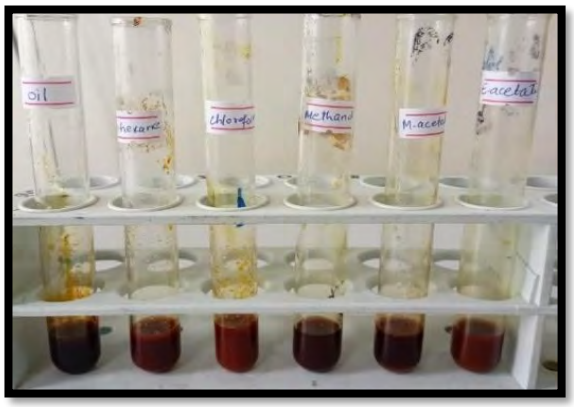
+: Present in extract/Oil, -: Absent in extract/Oil.

(a) Alkaloid

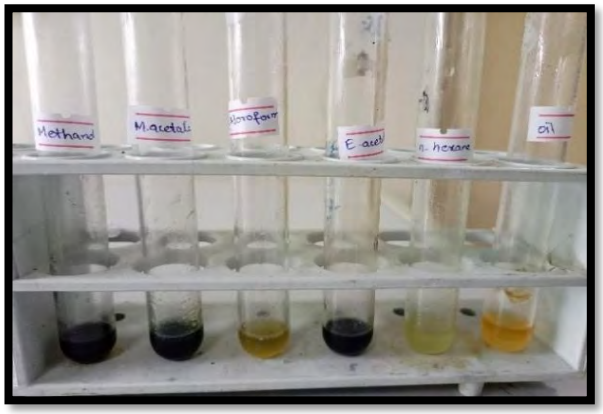
Mayer's Test



Wagner's Test

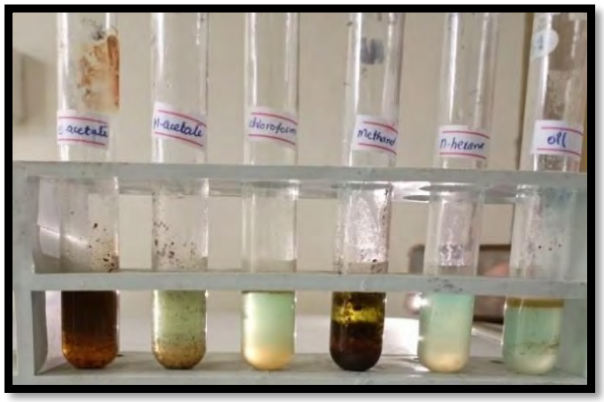


(b) Resins

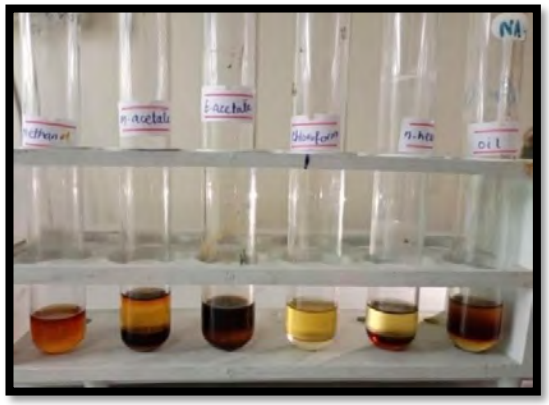


(c) Flavonoids

(d) Glycosides



(e) Steroids



(f) Saponin **(g) Tannins/ Phenol**

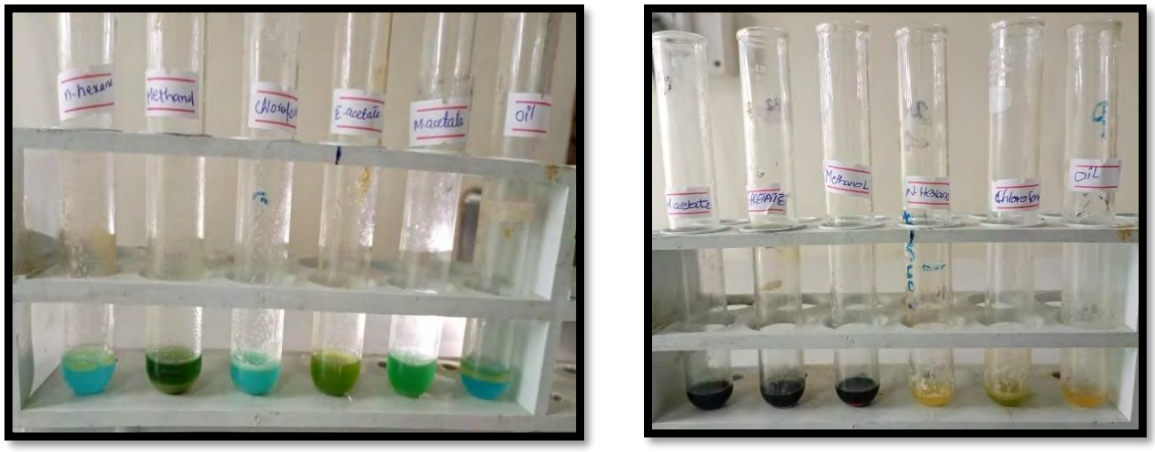


Fig. 4.3. Qualitative analysis of (a) alkaloid, (b) resins, (c) flavonoids, (d) glycosides, (e) steroids (f) saponin and (g) tannins/ phenols in *R. communis* extracts and oil

4.4. FTIR analysis of *R. communis L* extracts and oil

In addition to conventional qualitative phytochemical assays, characterization of the extracts and oil was also performed by its analysis through FTIR spectra. FTIR spectroscopy is an analytical technique that is generally used to indicate the presence of different functional groups in different types of materials (Organic, inorganic, or polymeric). FTIR spectra of extracts and oil indicate that a broad range of different compounds like amide linkages, ester and ether linkages, carbon-carbon, aromatic functional groups, carbon hydrogen bonds, and carbonyl linkages are present that confirm the presence of cellulose, lignin, and hemicellulose components. The functional groups present in extracts and oil are listed below in tables.

4.4.1 FTIR analysis of *R. communis L* methanolic extract

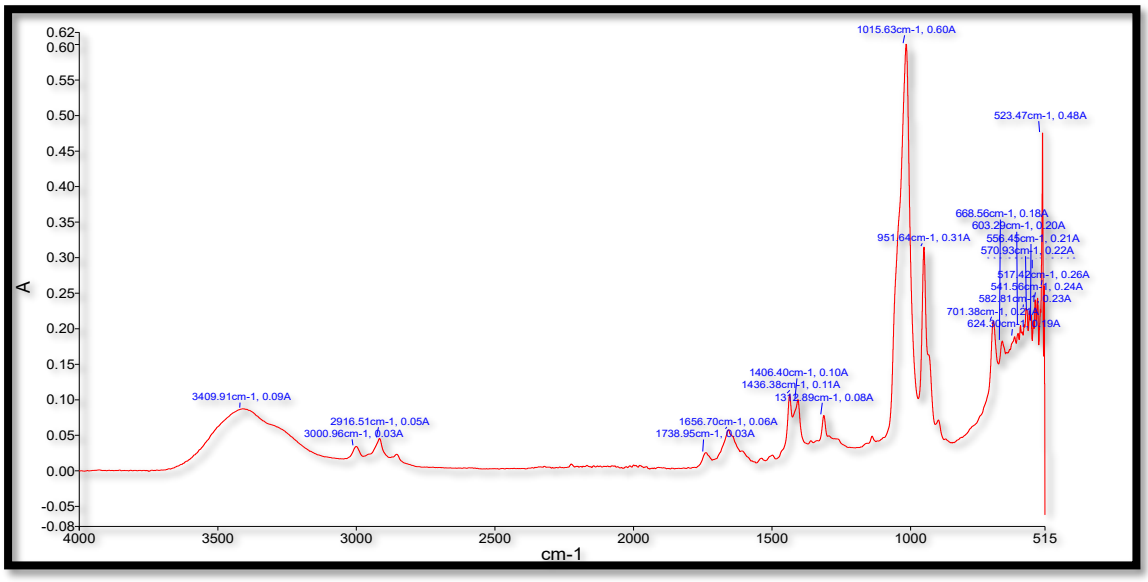


Fig. 4.4. FTIR spectrum obtained for *R. communis L* methanolic extract in the range of 4000-400 cm^{-1}.

Table 4.3. Stretches in the FTIR spectrum that correspond to functional groups in the methanolic extract of *R. communis L* pressed seed cake.

Band Positions (cm^{-1})	**Inferences of FTIR Spectrum**
3409	Carboxylic acid OH stretch, N-H stretch, Alcohol OH stretch
3000	=C-H stretch =C-H stretch
2916	-C-H stretch
1656	C=O, C=N, C=C stretches
1436, 1406	CH_3, CH_2
1312	CH_3
1015, 951	C-OH
701, 668, 624	CH out of plane bending (Carbohydrate)

4.4.2. FTIR analysis of *R. communis L* n-Hexane extract

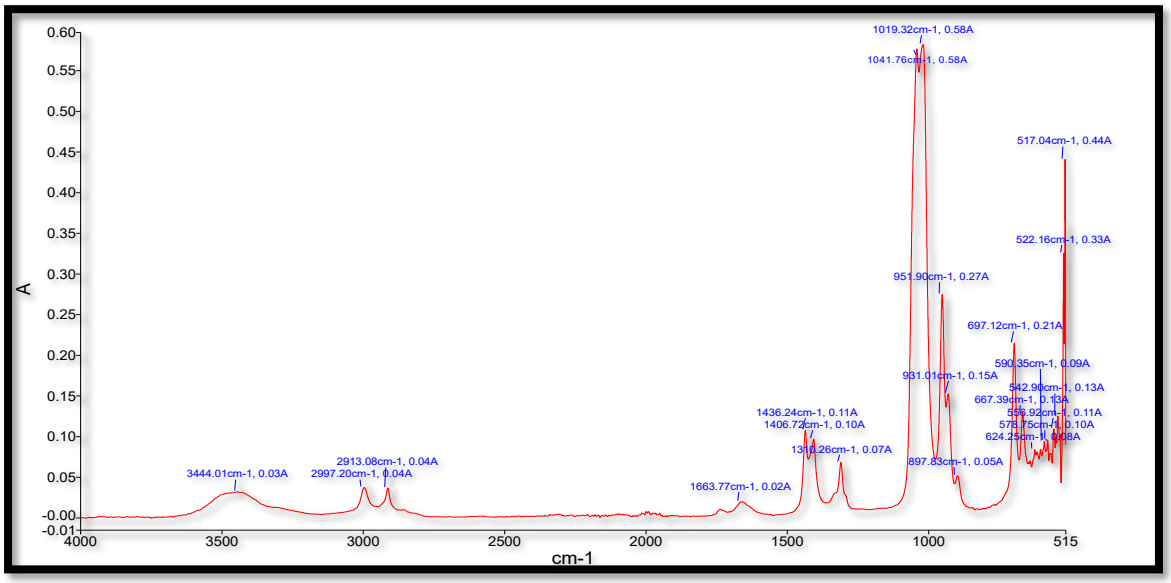


Fig. 4.5. FTIR spectrum obtained for *R. communis L* n-Hexane extract in the range of 4000-400 cm^{-1}.

Table 4.4. Stretches in the FTIR spectrum that correspond to functional groups in n-Hexane extract of *R. communis L* pressed seed cake

Band Positions (cm^{-1})	**Inferences of FTIR Spectrum**
3444	Carboxylic acid OH stretch, N-H stretch, Alcohol OH stretch
2997	=C-H stretch
2913	-C-H stretch
1663	C=C (medium-intensity alkene stretches)
1436	CH_3, CH_2
1406	CH3
1310	CH_3, NO_2 stretches
1041	C-OH
951	C-OH
897, 697, 590	CH out of plane bending (Carbohydrate)

4.4.3. FTIR analysis of *R. communis L* methyl acetate extract

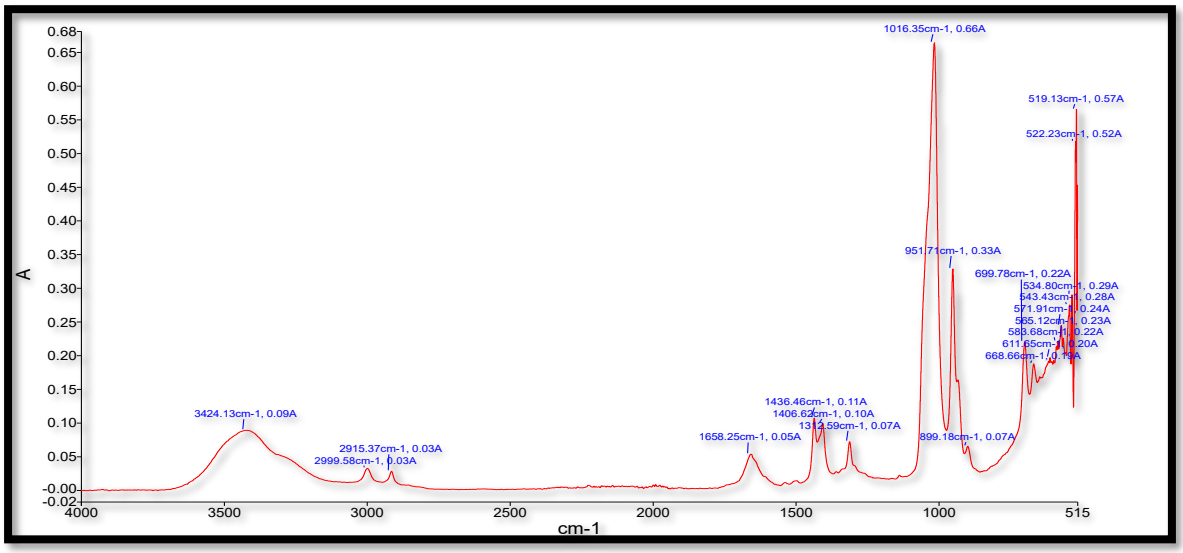


Fig. 4.6. FTIR spectrum obtained for *R. communis L* methyl acetate extract in the range of 4000-400 cm^{-1}

Table 4.5. Stretches in the FTIR spectrum that correspond to functional groups methyl acetate extract of *R. communis L* pressed seed cake

Band Positions (cm^{-1})	**Inferences of FTIR Spectrum**
3424	Carboxylic acid OH stretch, N-H stretch, Alcohol OH stretch
2999	=C-H stretch
2915	-C-H stretch
1658	C=O, C=N, C=C stretches
1436	CH_3, CH_2
1406	CH_3, CH_2
1312	CH_3
1016	C-OH
951	C-OH
699	CH out of plane bending (Carbohydrate)

4.4.4 FTIR analysis of *R. communis L* chloroform extract

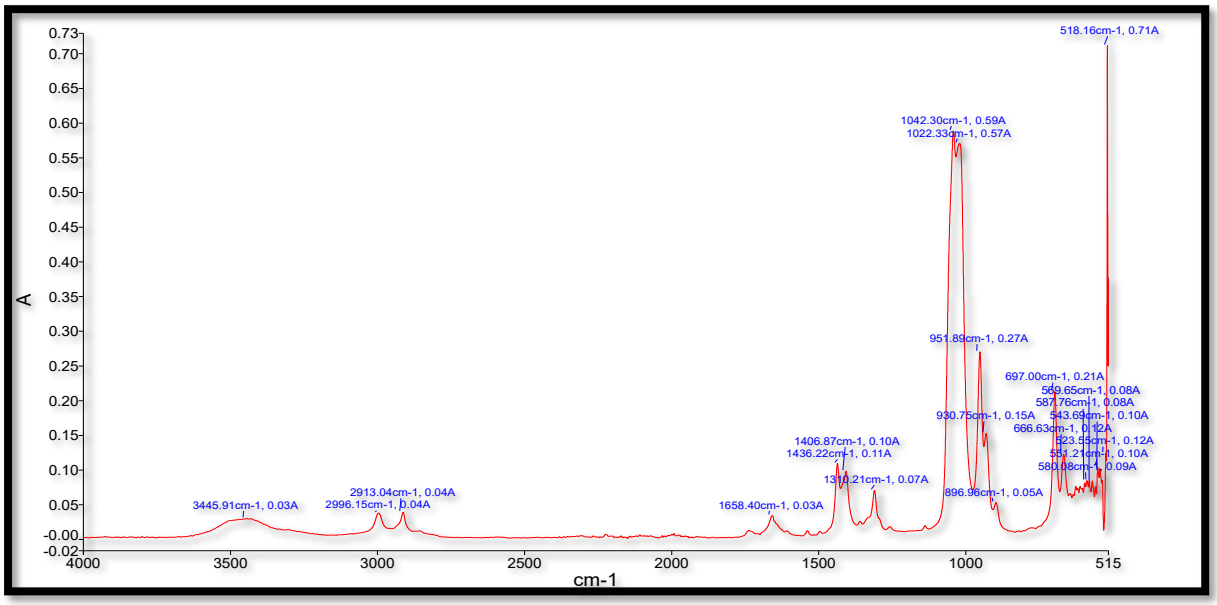


Fig. 4.7. FTIR spectrum obtained for *R. communis L* chloroform extract in the range of 4000-400 cm^{-1}.

Table 4.6. Stretches in the FTIR spectrum that correspond to functional groups in chloroform extract of *R. communis L* pressed seed cake

Band Positions (cm^{-1})	Inferences of FTIR Spectrum
3445	Carboxylic acid OH stretch, N-H stretch, Alcohol OH stretch
2996	=C-H stretch
2913	-C-H stretch
1658	C=O, C=N, C=C stretches
1436	CH_3, CH_2
1406	CH_3, CH_2
1310	CH_3
1042	C-OH
951	C-OH
930,896, 697	CH out of plane bending (Carbohydrate)

4.4.5. FTIR analysis of *R. communis L* ethyl acetate extract

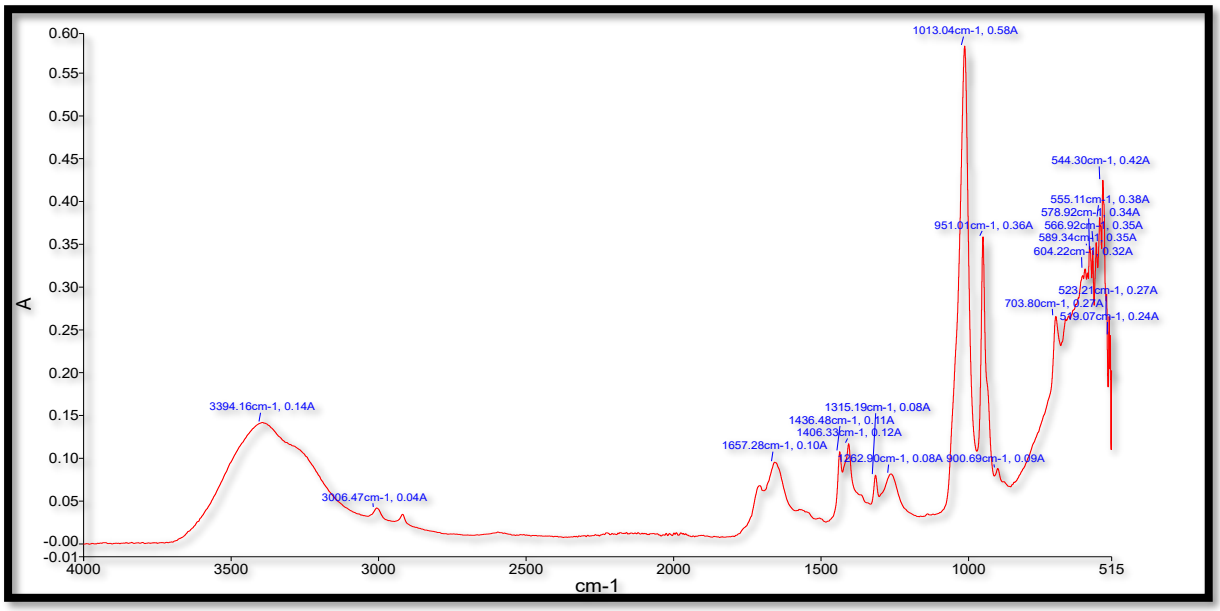


Fig. 4.8. FTIR spectrum obtained for *R. communis L* ethyl acetate extract in the range of 4000-400 cm^{-1}.

Table 4.7. Stretches in the FTIR spectrum that correspond to functional groups in ethyl acetate extract of *R. communis L* pressed seed cake

Band Positions (cm^{-1})	Inferences of FTIR Spectrum
3394	Carboxylic acid OH stretch, N-H stretch, Alcohol OH stretch
3006	=C-H stretch
1667	C=O, C=N, C=C stretches
1436	CH_3, CH_2
1406	CH_3, CH_2
1315	CH_3
1013, 951	C-OH
900, 703	CH out of plane bending (Carbohydrate)

4.4.6. FTIR analysis of *R. communis L* oil

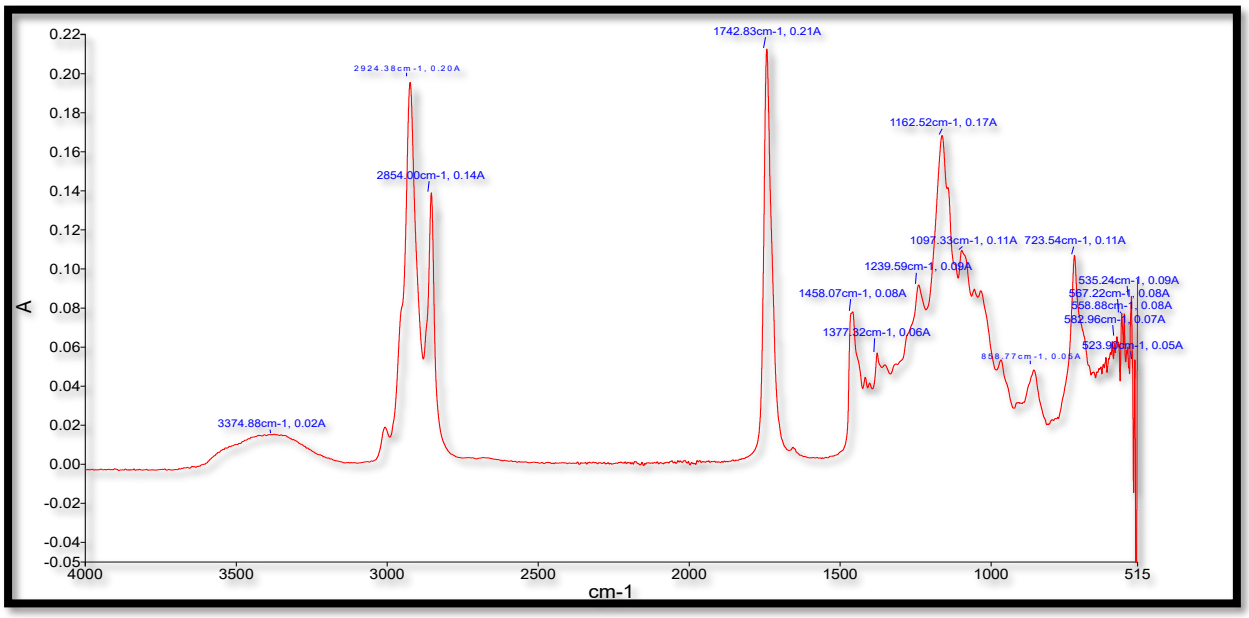


Fig. 4.9. FTIR spectrum obtained for *R. communis L* oil in the range of 4000-400 cm^{-1}.

Table 4.8. Stretches in the FTIR spectrum that correspond to functional groups in *R. communis L* oil

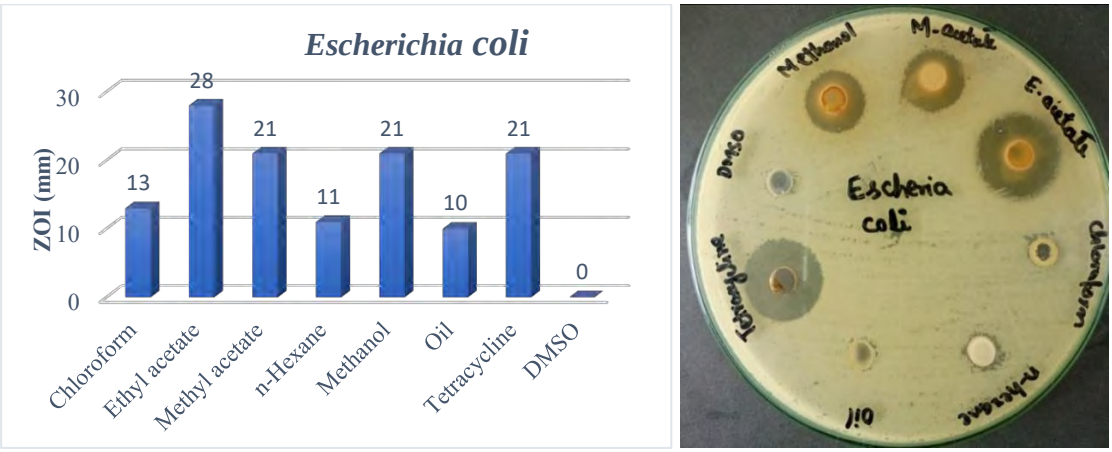
Band Positions (cm^{-1})	**Inferences of FTIR Spectrum**
3374	=C-H stretch
2924	-C-H stretch
2854	-C-H stretch
1742	C=O Ketone stretches
1458	CH_3, CH_2
1377	CH_3, NO_2
1162	C-OH
1097	C-OH
723, 582, 558, 523	CH out of plane bending (Carbohydrate)

4.5. Antibacterial Assay

In the current study, extracts and oil of castor seeds were utilized to check their antibacterial activity against gram-positive and gram-negative bacterial strains. ATCC and MDR were used to find out antimicrobial potential of castor. For this purpose, the agar well diffusion method was utilized. The bacterial suspension was spread over the agar plate and 100 microliter extract was added in each well and its activity was measured in millimeters. Crude extracts and Oil showed promising results in the inhibition of bacterial growth. Methanolic, methyl acetate, and ethyl acetate extracts possessed better antimicrobial activity against all the selected ATCC and MDR bacterial strains. *Ricinus communis L* chloroform, and n-Hexane extract however showed moderate to low activity. Oil only showed activity against *Escherichia coli.* Tetracycline, a broad-spectrum antibiotic, was used as a positive control and depicted great antibacterial activity against all strains while DMSO was used as a negative control.

4.5.1. Antibacterial activity of extracts and oil against *Escherichia coli*

E. coli, a gram- negative bacterium, was susceptible to all extracts. Ethyl acetate showed the highest activity against *E. coli* with a ZOI of 28mm followed by methanolic, methyl acetate, and chloroform which exhibit zones of inhibition of 21mm, 21mm, and 13mm respectively. Castor oil and n-Hexane extracts showed the least activity against bacterial strain and formed zones of inhibition 10 and 11mm respectively. Tetracycline, as a positive control, formed 21mm ZOI and DMSO showed no antibacterial activity.

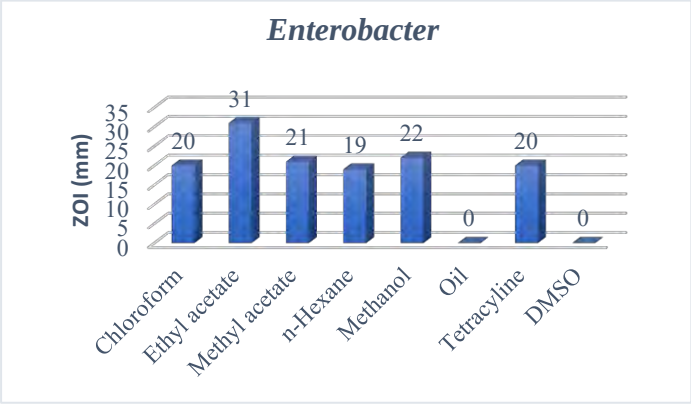


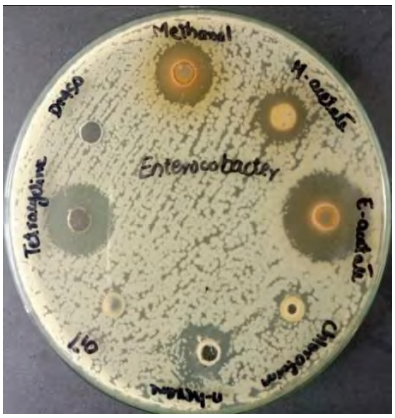
(a) **(b)**

Fig. 4.10. Antibacterial activity of extracts and oil against *Escherichia coli:* (a) Graph showing the ZOI of different extracts against *Escherichia coli* (b) Diagram showing the antibacterial exhibited by *R. communis L* oil and different extracts against *Escherichia coli.*

4.5.2. Antibacterial Activity of extracts and oil against *Enterobacter*

Enterobacter a Gram-negative bacterium was most susceptible to ethyl acetate, methanolic, methyl acetate extracts, and chloroform and these extracts inhibited the growth of strain to the maximum extent by showing ZOI 31, 22, and 21mm, 20mm respectively, while n-Hexane (19mm) extract of seed cake had a medium effect on the growth of *Enterobacter*. Castor oil showed no activity against *Enterobacter*. Tetracycline (positive control) showed 25mm ZOI. DMSO (negative control) showed no activity against *Enterobacter.*



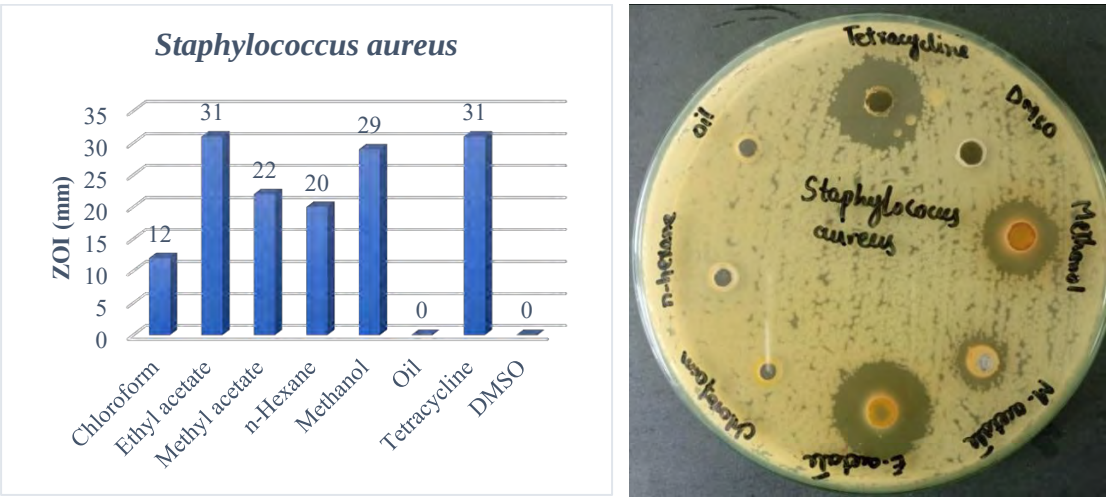


(a) **(b)**

Fig. 4.11. Antibacterial activity of extracts and oil against *Enterobacter* (a): Graph showing the ZOI of different extracts against *Enterobacter* (b): Diagram showing the antibacterial exhibited by *R. communis L* oil and different extracts against *Enterobacter.*

4.5.3. Antibacterial Activity of extracts and oil against *Staphylococcus aureus*

Staphylococcus aureus is a Gram-positive bacterium. Ethyl acetate and methanolic extracts of castor showed the highest activity with ZOI 31mm and 29mm respectively. Castor oil didn't show any activity against *S. aureus* while methyl acetate and n-Hexane showed moderate activity with ZOI 22mm and 20mm respectively. Chloroform showed low activity with 12 mm ZOI. Tetracycline showed high activity against *Staphylococcus aureus* with ZOI 31mm. DMSO showed no activity against *Staphylococcus aureus.*

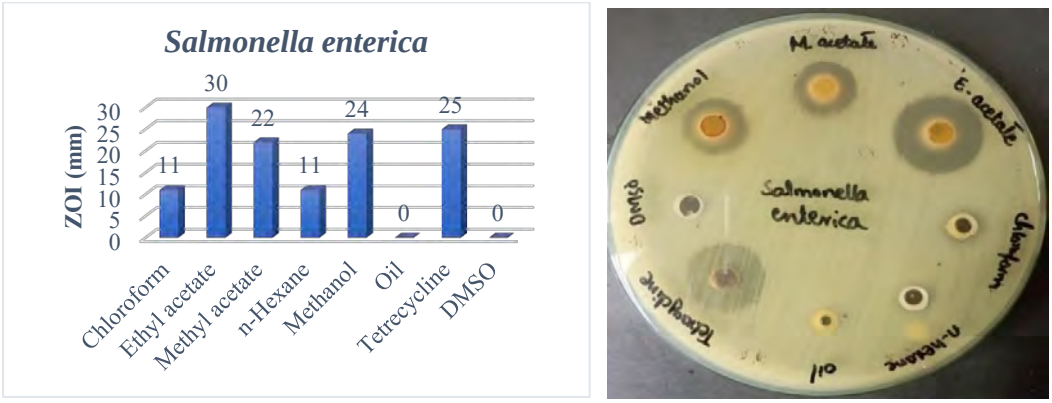


(a) **(b)**

Fig. 4.12. Antibacterial activity of extracts and oil against *Staphylococcus aureus:* (a): Graph showing the ZOI of different extracts against *Staphylococcus aureus* (b): Diagram showing the antibacterial exhibited by *R. communis L* oil and different extracts against *Staphylococcus aureus.*

4.5.4. Antibacterial activity of extracts and oil against *Salmonella enterica*

Salmonella enterica, a Gram-negative bacterium. Ethyl acetate showed the highest activity (30mm). Methanolic and methyl acetate extracts formed good ZOI, which were 24mm and 22mm respectively. Castor oil didn't show any activity against *S. enterica.* Chloroform and n-Hexane both extracts showed activity with ZOI 11mm individually. Tetracycline showed 25mm ZOI. *Salmonella enterica* was susceptible to the antibiotic. DMSO didn't show any activity.

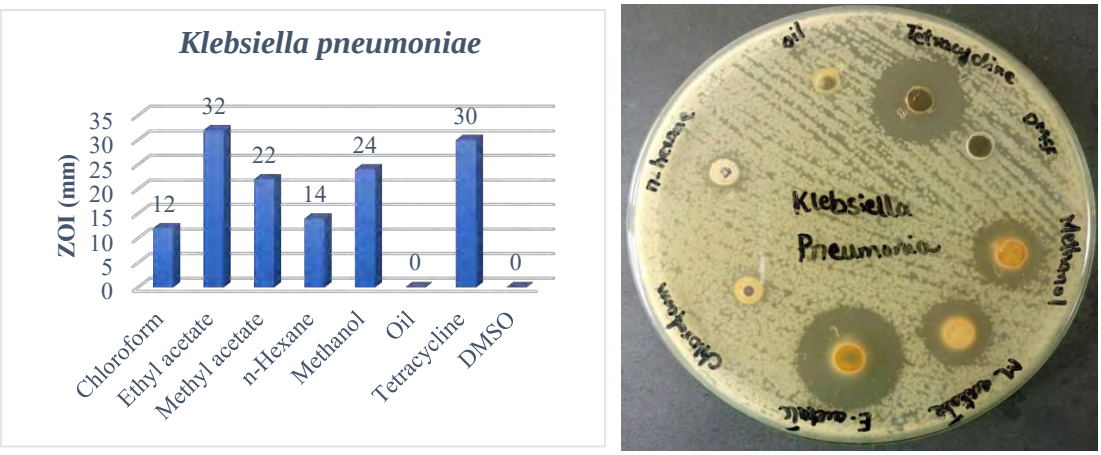


(a) **(b)**

Fig. 4.13. Antibacterial activity of extracts and oil against *Salmonella enterica:* (a): Graph showing the ZOI of different extracts against *Salmonella enterica* (b): Diagram showing the antibacterial exhibited by *R. communis L* oil and different extracts against *Salmonella enterica.*

4.5.5. Antibacterial activity of extracts and oil against *Klebsiella pneumoniae*

Klebsiella pneumoniae is a Gram-negative bacterium. Ethyl acetate and methanolic extracts showed high activity against *K. pneumoniae* with ZOI 32mm and 24mm respectively. Methyl acetate extract showed moderate activity (22mm). Castor oil didn't show any activity. n-Hexane and chloroform showed 14mm and 12mm ZOI 12mm against *K. pneumoniae.* Tetracycline used as positive control showed the highest activity against *K. pneumoniae* with ZOI 30mm. DMSO showed no activity against *K. pneumoniae.*

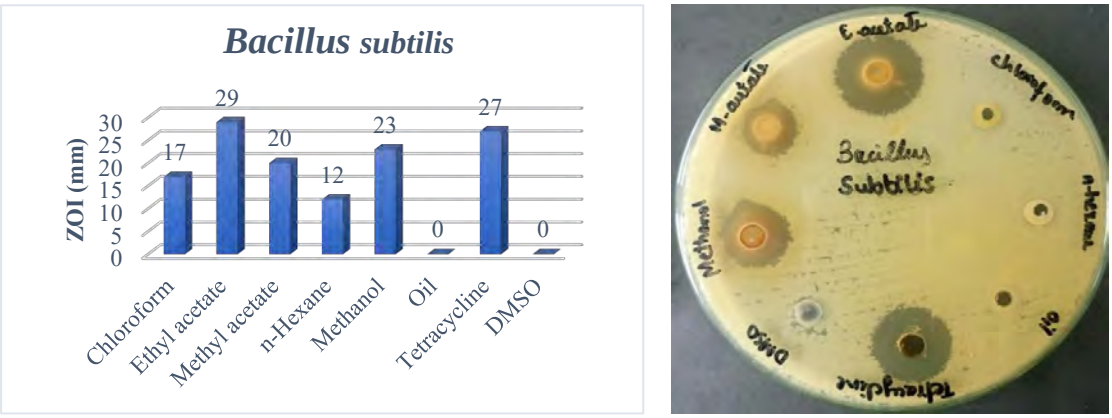


(a) **(b)**

Fig. 4.14. Antibacterial activity of extracts and oil against *Klebsiella pneumoniae* (a): Graph showing the ZOI of different extracts against *Klebsiella pneumoniae* (b): Diagram showing the antibacterial exhibited by *R. communis L* oil and different extracts against *Klebsiella pneumoniae.*

4.5.6. Antibacterial activity of extracts and oil against *Bacillus subtilis*

Bacillus subtilis, a Gram-positive bacterium was most susceptible to the ethyl acetate extract which showed a ZOI of 29mm. Methanolic extract of castor showed good activity with ZOI 23mm, castor oil didn't show any activity. Chloroform and methyl acetate inhibited growth with ZOI, 17mm and 20mm respectively. n-Hexane extract of castor showed low activity against *B. subtilis* with ZOI 12mm. DMSO was used as a negative control against *B. subtilis.* Tetracycline showed activity against *B. subtilis* with ZOI 20mm.

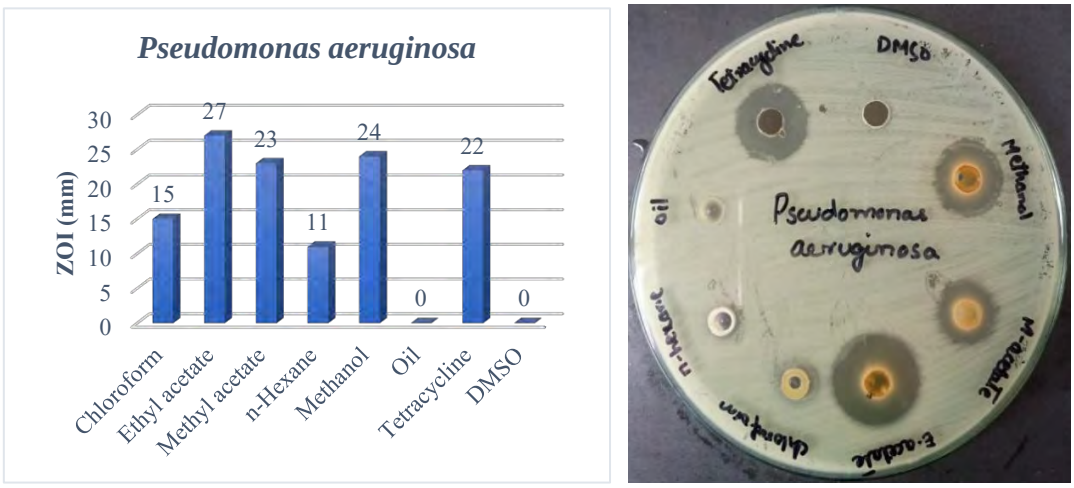


(a) **(b)**

Fig. 4.15. Antibacterial activity of extracts and oil against *Bacillus subtilis:* (a): Graph showing the ZOI of different extracts against *Bacillus subtilis* (b): Diagram showing the antibacterial exhibited by *R. communis L* oil and different extracts against *Bacillus subtilis.*

4.5.7. Antibacterial activity of extracts and oil against *Pseudomonas aeruginosa*

Ethyl acetate and methanolic extracts showed the highest activity with ZOI 27mm and 24mm respectively. Methyl acetate showed 23mm ZOI. Castor oil didn't show any activity against *Pseudomonas aeruginosa*. Chloroform extracts formed moderate activities with ZOI 15mm respectively. n-Hexane showed low activity (11mm). Tetracycline was used as positive control and it showed 22mm ZOI. DMSO showed no activity against *Pseudomonas aeruginosa.*

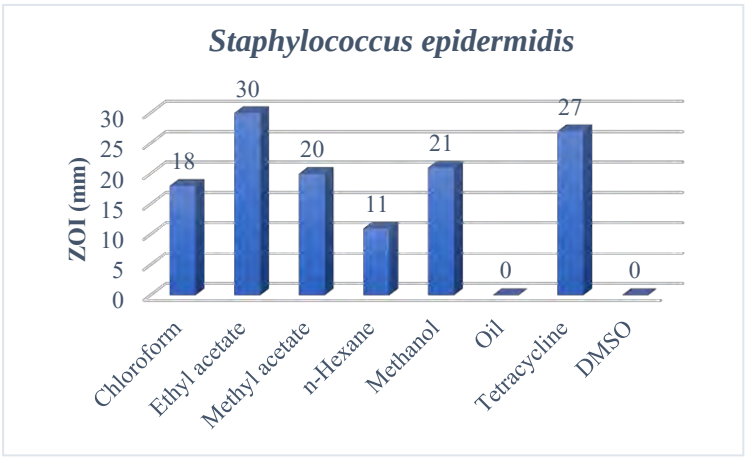


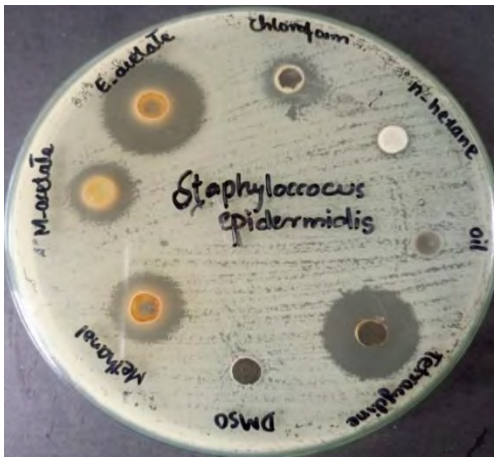
(a) **(b)**

Fig. 4.16. Antibacterial activity of extracts and oil against *Pseudomonas aeruginosa:* (a): Graph showing the ZOI of different extracts against *Pseudomonas aeruginosa* (b): Diagram showing the antibacterial exhibited by *R. communis L* oil and different extracts against *Pseudomonas aeruginosa.*

4.5.8. Antibacterial activity of extracts and oil against *Staphylococcus epidermidis*

Staphylococcus epidermidis, a Gram-positive bacterium was most susceptible to ethyl acetate and methanolic extracts with ZOI 30mm and 21mm respectively. Methyl acetate extract showed good activity with ZOI 20mm, Castor oil showed no activity. Chloroform and n-Hexane showed moderate to low activities with ZOI 18mm and 11mm respectively. The strain was susceptible to antibiotic tetracycline (27mm) and DMSO showed no activity against *S. epidermidis.*





(a) **(b)**

Fig. 4.17. Antibacterial activity of extracts and oil against *Staphylococcus epidermidis:* (a): Graph showing the ZOI of different extracts against *Staphylococcus epidermidis* (b): Diagram showing the antibacterial exhibited by *R. communis L* oil and different extracts against *Staphylococcus epidermidis.*

Table 4.9. Relative activity of extracts and oil against ATCC bacterial strains

The following table shows relative activities of castor seed extracts and oil against ATCC bacterial strains.

Strains	Extracts/drug and Oil							
	Ethyl acetate	**Methanol**	**Methyl acetate**	**Chloroform**	**n-Hexane**	**Oil**	**Tetracycline**	**DMSO**
E. coli	High	High	High	Moderate	Low	Low	High	-
S. epidermidis	High	High	High	Moderate	Low	-	High	-
B. subtilis	High	High	High	Moderate	Low	-	High	-
K. pneumoniae	High	High	High	Low	Moderate	-	High	-
P. aeruginosa	High	High	High	Moderate	Low	-	High	-
Enterobacter	High	High	High	High	Moderate	-	High	-
S. enterica	High	High	High	Low	Low	-	High	-
S. aureus	High	High	High	Low	High	-	High	-

4.6. MIC values of *R. communis* extracts and oil against ATCC bacterial strains

MIC is the minimum inhibitory concentration of extract/drug and oil at which strain showed 80% growth reduction. Ethyl acetate and Methyl acetate showed 0.05mg/ml and MIC 0.5mg/ml values against all selected ATCC strains. Chloroform showed 50-0.5mg/ml MIC range. Methanol demonstrated 0.5-0.05mg/ml value. n-Hexane showed 500-0.5mg/ml range. Oil showed 500mg/ml MIC value against *E. coli.* Tetracycline showed 0.5-0.05mg/ml MICs range.

Table 4.10. MIC values of *R. communis* extracts and oil against ATCC bacterial strains

Extracts/ drug and oil	MIC (mg/ml)							
	ATCC bacterial Strains							
	E. coli	*S. epidermidis*	*B. subtilis*	*K. pneumoniae*	*Enterobacter*	*S. enterica*	*S. aureus*	*P. aeruginosa*
Chloroform	50	5	5	50	0.5	50	50	5
Ethyl acetate	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Methyl acetate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Methanol	0.5	0.5	0.5	0.5	0.5	0.5	0.05	0.5
n-Hexane	500	500	50	5	0.5	50	0.5	50
Oil	500	-	-	-	-	-	-	-
Tetracycline	0.5	0.05	0.05	0.05	0.5	0.5	0.05	0.5

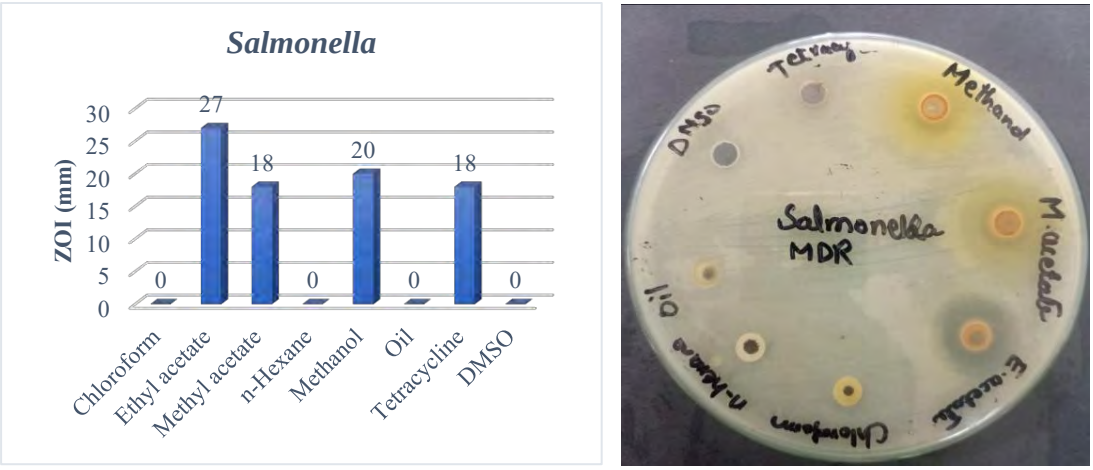
4.7. Antibacterial activity against MDR strains

Public health is severely hampered by MDR bacterial strains like *Salmonella*, *K. pneumoniae*, and *P. aeruginosa*. Because these bacteria have built up resistance to numerous antibiotics, infections caused by them are more challenging to treat. Severe gastrointestinal symptoms can result from the common food borne pathogen *Salmonella*. Infections of the urinary and respiratory tracts can be brought on by *K. pneumoniae*, which is frequently encountered in healthcare facilities. *P. aeruginosa* is frequently identified in hospitals and is known to cause a variety of illnesses, including skin and respiratory infections. To protect the public's health, dealing with MDR strains necessitates a multifaceted strategy that includes the creation of new antibiotics and the adoption of infection prevention and control methods. Plant based antimicrobial compounds are the best option to deal with MDR strains.

Phytochemicals can be used as an alternative to antibiotics because of their diversity on the metabolic, genetic, and physiological fronts, as well as the rapid evolution of resistant microorganisms and the absence of tactile management. By doing their antagonistic effects on bacterial membrane proteins, efflux pumps, biofilms, and bacterial cell-to-cell communications—all of which are significant contributors to the emergence of drug resistance—many phytochemicals have successfully demonstrated their inhibitory potential against MDR pathogens. The development of bacterial resistance against the complex mixture of phytochemical components found in plant extracts is rather purposeful (Suganya et al., 2022).

4.7.1. Antibacterial activity of extracts and oil against MDR strain *Salmonella*

Ethyl acetate showed the highest activity (27mm). Methanolic and methyl acetate extracts formed good ZOI, which were 20mm and 18mm respectively. Castor oil, chloroform, n-Hexane and DMSO didn't show any activity against *Salmonella.* Tetracycline showed 18mm ZOI.

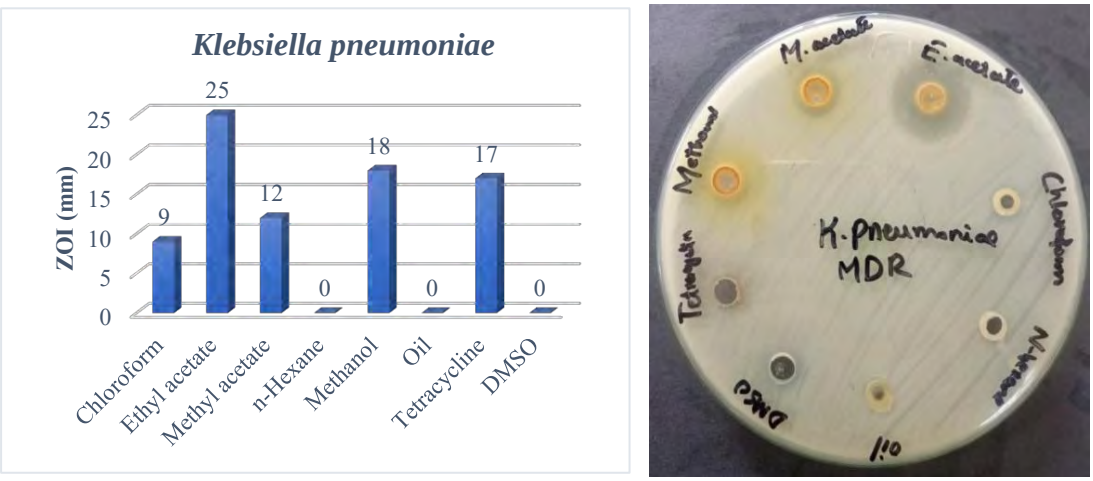


(a) **(b)**

Fig. 4.18. Antibacterial activity of extracts and oil against MDR strain *Salmonella*: (a): Graph showing the ZOI of different extracts against *Salmonella* (b): Diagram showing the antibacterial exhibited by *R. communis L* oil and different extracts against *Salmonella.*

4.7.2. Antibacterial Activity of extracts and oil against MDR strain *Klebsiella pneumoniae*

Ethyl acetate and methanolic extracts showed high activity against *K. pneumoniae* with ZOI 25mm and 18mm respectively. Methyl acetate extract showed 12mm ZOI. n-Hexane, oil and DMSO didn't show any activity. Chloroform showed 9mm ZOI. Tetracycline used as positive control showed ZOI 17mm.

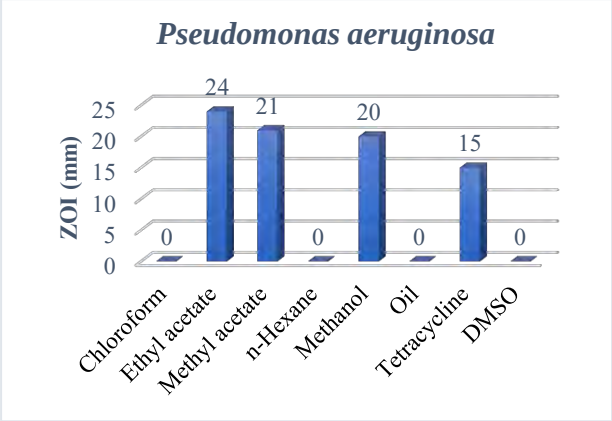


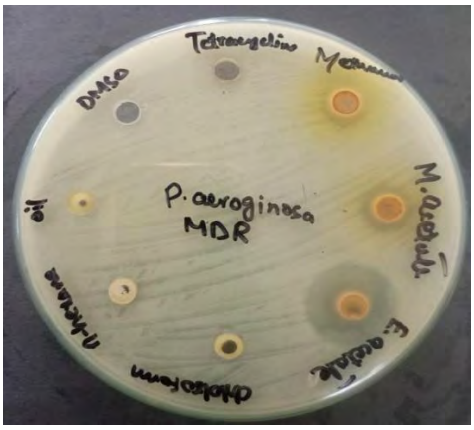
(a) **(b)**

Fig. 4.19. Antibacterial activity of extracts and oil against MDR strain *Klebsiella pneumoniae* (a): Graph showing the ZOI of different extracts against *Klebsiella pneumoniae* (b): Diagram showing the antibacterial exhibited by *R. communis L* oil and different extracts against *Klebsiella pneumoniae.*

4.7.3. Antibacterial Activity of extracts and oil against MDR strain *Pseudomonas aeruginosa*

Ethyl acetate, methyl acetate and methanolic extracts showed the highest activity with ZOI 24mm, 21mm and 20mm respectively. Chloroform, n-Hexane, oil and DMSO didn't show any activity against MDR *Pseudomonas aeruginosa*. Tetracycline was used as positive control and it showed 15mm ZOI.





(a) **(b)**

Fig. 4.20. Antibacterial activity of extracts and oil against MDR strain *Pseudomonas aeruginosa:* (a): Graph showing the ZOI of different extracts against *Pseudomonas aeruginosa* (b): Diagram showing the antibacterial exhibited by *R. communis L* oil and different extracts against *Pseudomonas aeruginosa.*

Table 4.11. Relative activity of extracts and oil against MDR bacterial strains

The following table shows relative activities of castor seed extracts and oil against MDR bacterial strains.

Strains	Extracts and Oil							
	Ethyl acetate	**Methanol**	**Methyl acetate**	**Chloroform**	**n-Hexane**	**Oil**	**Tetracycline (+)**	**DMSO (-)**
P. aeruginosa	High	High	High	-	-	-	Moderate	-
K. pneumoniae	High	Moderate	Moderate	Low	-	-	High	-
Salmonella	High	High	Moderate	-	-	-	High	-

4.8. MIC values of *R. communis* extracts and oil against MDR bacterial strains

MICs values were determined against three MDR bacterial strains. Ethyl acetate and Methyl acetate showed 0.05-0.5mg/ml and MIC 5-50mg/ml values against all selected strains. Chloroform showed 50mg/ml MIC against *K. pneumoniae*. Methanol demonstrated 0.5mg/ml values. n-Hexane and oil didn't show MICs. Tetracycline showed 5-0.5mg/ml MICs range.

Table 4.12. MIC values of *R. communis* extracts and oil against MDR bacterial strains

Extracts/drug and oil	MDR Strains		
	MIC (mg/ml)		
	Salmonella	***K. pneumoniae***	***P. aeruginosa***
Chloroform	-	50	-
Ethyl acetate	0.05	0.5	0.5
Methyl acetate	5	50	0.5
Methanol	0.5	0.5	0.5
n-Hexane	-	-	-
Oil	-	-	-
Tetracycline	5	0.5	5

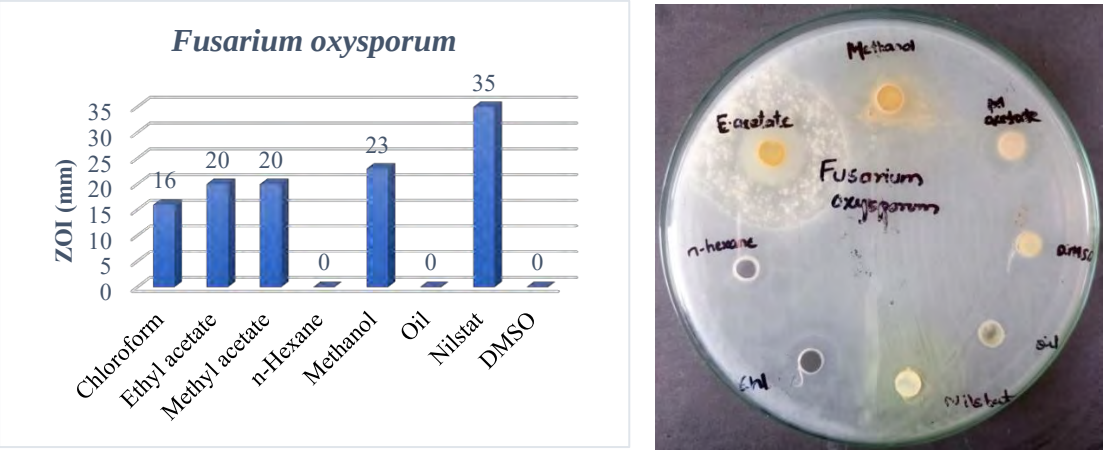
4.9. Antifungal Activity

Castor seeds extracts and oil were tested for their antifungal activities in the current investigation. The agar well diffusion method was followed. The fungal suspension was spread on agar plate, 100 microliters of extracts and oil were placed in the wells, and millimeters of activity were recorded. The suppression of fungal development by crude extracts of pressed seed cake and oil exhibited encouraging results. The antifungal potential of *Ricinus communis L* pressed seed cake extracts, and seed oil was examined against five clinical selected strains: *Candida albicans, Aspergillus flavus, Aspergillus niger, Fusarium oxysporum,* and *Curvularia lunata* and three phytopathogenic strains *Aspergillus flavus, Fusarium* and *Penicillium chrysogenum.* Extracts demonstrated high activity against selected strains.

Antifungal activity of extracts and seed oil against clinical fungal strains

4.9.1. Antifungal activity of extracts and seed oil against *Fusarium oxysporum*

The filamentous fungal specie, *Fusarium oxysporum* is a member of the genus Fusarium. *Fusarium oxysporum* is a pathogen for plants as well as for human beings. Fusarium oxysporum, a widespread soil-borne pathogen that ranks fifth among the top 10 fungal plant infections, produces devastating vascular disease in more than 100 plant species. It has now been identified as a human pathogen that infects patients with impaired immune systems (Husaini et al., 2018). Methanolic extract showed the highest activity against *Fusarium oxysporum* with a ZOI of 23mm followed by methyl acetate and ethyl acetate which showed ZOI of 20mm each. Castor oil and n-Hexane didn't show any activity. Chloroform showed moderate activity with a ZOI of 16mm. Nilstat as positive control showed a ZOI 35mm and DMSO showed no activity.

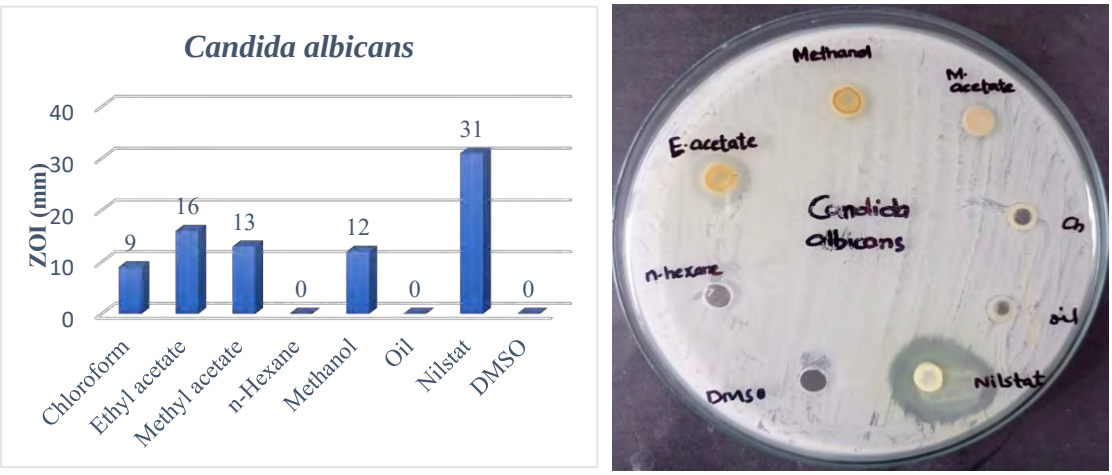


(a) **(b)**

Fig. 4.21. Antifungal activity of extracts and oil against *Fusarium oxysporum:* (a): Graph showing the ZOI of different extracts against *Fusarium oxysporum* (b): Diagram showing the antifungal activity exhibited by *R. communis L* and different extracts against *Fusarium oxysporum.*

4.9.2. Antifungal activity of extracts and seed oil against *Candida albicans*

Candida albicans is an opportunistic human fungal pathogen. *C. albicans*, despite being a typical part of our gut flora, has the ability to colonize almost all human tissues and organs, and cause invasive, dangerous infections (Kim & Sudbery, 2011). Ethyl acetate extract showed the highest activity against *Candida albicans* with ZOI 16mm. Methyl acetate and methanolic extracts showed moderate activity against *Candida albicans* with ZOI 13mm respectively. Chloroform showed less activity with ZOI 9mm. n-Hexane and Seed oil didn't show any activities against *C. albicans*. Nilstat as a positive control showed a ZOI 31mm and DMSO showed no activity.

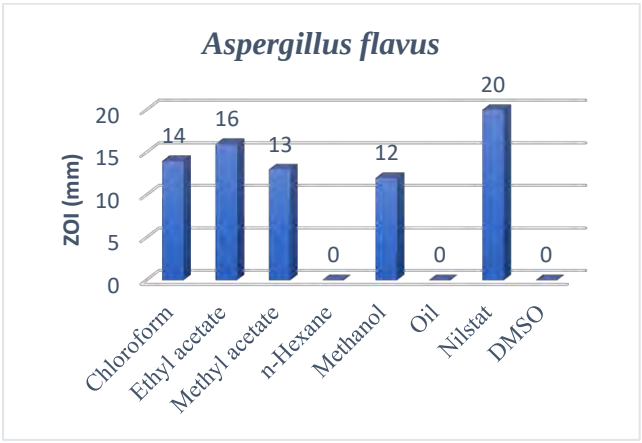


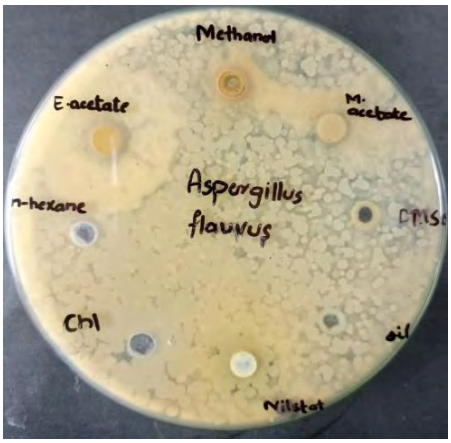
(a) **(b)**

Fig. 4.22. Antifungal activity of extracts and oil against *Candida albicans:* (a): Graph showing the ZOI of different extracts against *Candida albicans* (b): Diagram showing the antifungal activity exhibited by *R. communis L* oil and different extracts against *Candida albicans.*

4.9.3. Antifungal activity of extracts and seed oil against *Aspergillus flavus*

It is an opportunistic pathogen for humans, animals, and insects. It causes invasive and non-invasive aspergillosis in human, animals, and insects. People also experience allergic reaction to it (Amaike & Keller, 2011) (Tang et al., 2018). Methanol extract showed the highest activity against *Aspergillus flavus* with a ZOI of 20mm. Ethyl acetate and methyl acetate showed moderate activities with 16mm and 15mm ZOI. Chloroform extracts showed ZOI 14mm. n-Hexane and oil didn't show ZOI. Nilstat as positive control showed ZOI 20mm and DMSO showed no activity.



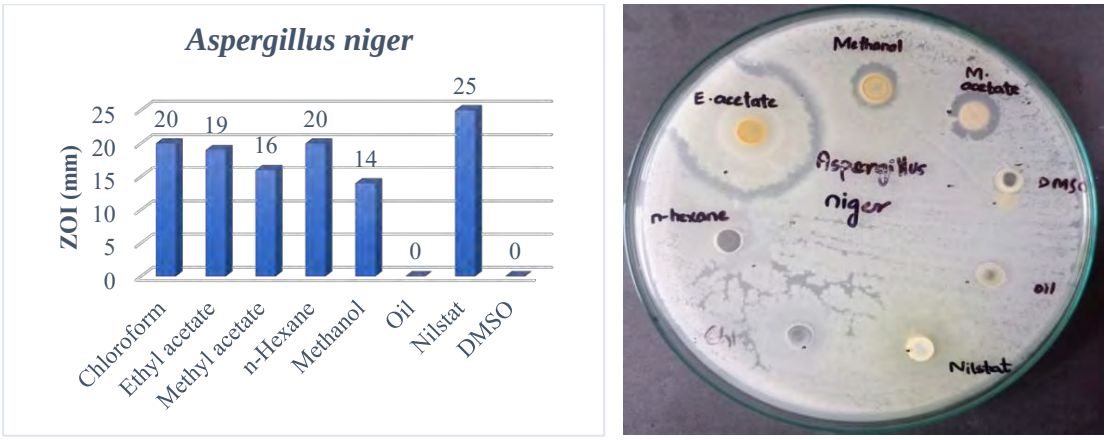


(a) **(b)**

Fig. 4.23. Antifungal activity of extracts and oil against *Aspergillus flavus:* (a): Graph showing the ZOI of different extracts against *Aspergillus flavus* (b): Diagram showing the antifungal activity exhibited by *R. communis L* oil and different extracts against *Aspergillus flavus.*

4.9.4. Antifungal activity of extracts and seed oil against *Aspergillus niger*

An opportunistic pathogen called *Aspergillus niger* can be isolated indoors as well as outdoors. *A. niger* spores can accumulate in the bronchioles of the human respiratory system because they are easily aerosolized (Erfandoust et al., 2020). n-Hexane and chloroform extracts showed the highest activities against *Aspergillus niger* with ZOI 20mm each. Ethyl acetate extract showed 19mm ZOI. Methyl acetate and methanolic extracts showed ZOI 16mm and 14mm respectively. *R. communis L* oil didn't show activity. Nilstat as positive control showed a ZOI of 25mm and DMSO showed no activity.

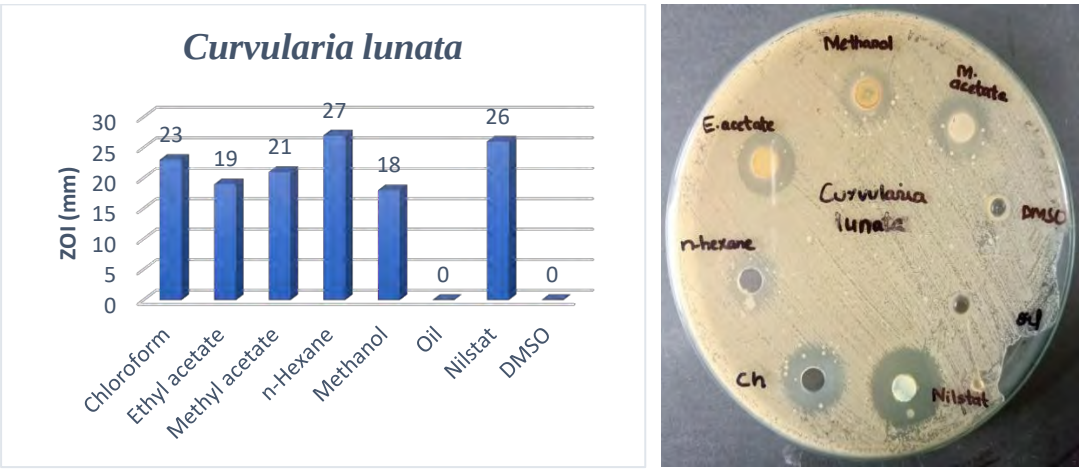


(a) **(b)**

Fig. 4.24. Antifungal activity of extracts and oil against *Aspergillus niger:* (a): Graph showing the ZOI of different extracts against *Aspergillus niger* (b): Diagram showing the antifungal activity exhibited by *R. communis L* oil and different extracts against *Aspergillus niger.*

4.9.5. Antifungal activity of extracts and seed oil against *Curvularia lunata*

Curvularia lunata can infect both people, plants and animals (Al-Odaini et al., 2022). n-Hexane extracts showed the highest activity against *Curvularia lunata* with ZOI 27mm. Chloroform and methyl acetate extracts showed ZOI 23mm and 21mm respectively. Ethyl acetate and Methanolic showed 19mm and18mm ZOI respectively. Castor oil didn't show any activity. Nilstat as a positive control showed a ZOI 26mm and DMSO showed no activity.



(a) **(b)**

Fig. 4.25. Antifungal activity of extracts and oil against *Curvularia lunata* (a): Graph showing the ZOI of different extracts against *Curvularia lunata* (b): Diagram showing the antifungal activity exhibited by *R. communis L* oil and different extracts against *Curvularia lunata.*

Table 4.13. Relative activity of extracts and oil against pathogenic fungal strains

The following table shows relative activities of castor seed extracts and oil against fungal strains.

Strains	Extracts/drug and oil							
	Methanol	**Methyl acetate**	**Ethyl acetate**	**n-Hexane**	**Chloroform**	**Oil**	**Nilstat (+)**	**DMSO (-)**
Fusarium oxysporum	High	High	High	-	Moderate	-	High	-
Candida albicans	Low	Low	Moderate	-	Low	-	High	-
Aspergillus flavus	High	Moderate	Moderate	-	Moderate	-	High	-
Aspergillus niger	Moderate	Moderate	Moderate	High	High	-	High	-
Curvularia lunata	Moderate	High	Moderate	High	High	-	High	-

4.10. MIC of *R. communis* seed extracts and oil for Clinical fungal strains

MIC is the minimum inhibitory concentration of extract/drug and oil at which fungal growth reduces 80%. Ethyl acetate and Methyl acetate showed MICs value ranges. 0.5-5mg/ml against all selected clinical strains. Chloroform showed 50-0.05mg/ml MIC range. Methanol demonstrated 50-0.5mg/ml value. n-Hexane showed 0.5-0.05mg/ml range. Oil didn't show activity against fungal strains. Nilstat showed 0.5-0.05mg/ml MICs.

Table 4.14. MIC of *R. communis L* seed extracts and oil for clinical fungal strains

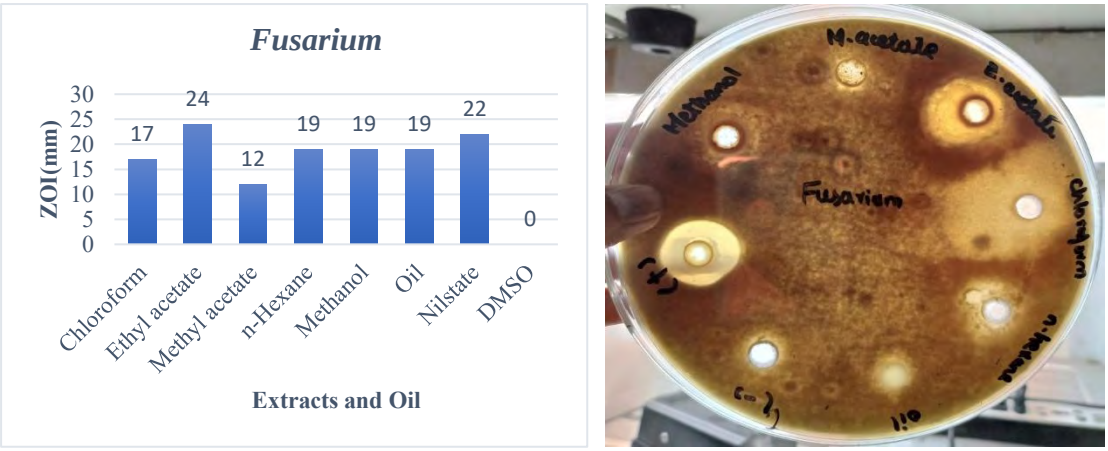
Extracts/drug and oil	Fungal Strains				
	MIC (mg/ml)				
	Fusarium oxysporum	***Candida albicans***	***Aspergillus flavus***	***Aspergillus niger***	***Curvularia lunata***
Chloroform	5	50	5	0.5	0.05
Ethyl acetate	0.5	0.5	0.5	5	5
Methyl acetate	0.5	0.5	0.5	5	0.5
Methanolic	0.5	50	50	5	5
n-Hexane	-	-	-	0.5	0.05
Oil	-	-	-	-	-
Nilstat	0.05	0.05	0.5	0.5	0.05

4.11. Antifungal activity of extracts and seed oil against Phytopathogenic Fungal Strains

The growing number of crop losses resulting from infection or contamination by pesticide-resistant pre- and post-harvest plant pathogenic fungi highlights the need for the development of fundamentally novel and secure antifungal techniques in agriculture (Tóth et al., 2020).

4.11.1. Antifungal activity of extracts and seed oil against *Fusarium*

There are hundreds of different species in the genus *Fusarium*. In contrast to others in the genus that have a wide host range, certain plant pathogenic individuals are limited to a single host species. This genus is one of the most damaging plant pathogenic fungi, and it can cause significant financial losses in agriculture (Al-Hatmi et al., 2019). Ethyl acetate showed 24mm ZOI. Methyl acetate and Chloroform showed 12 and 17mm ZOI respectively. Methanol, n-Hexane, and oil showed 19mm ZOIs individually. Negative control didn't show any activity while positive control showed 22mm ZOI.

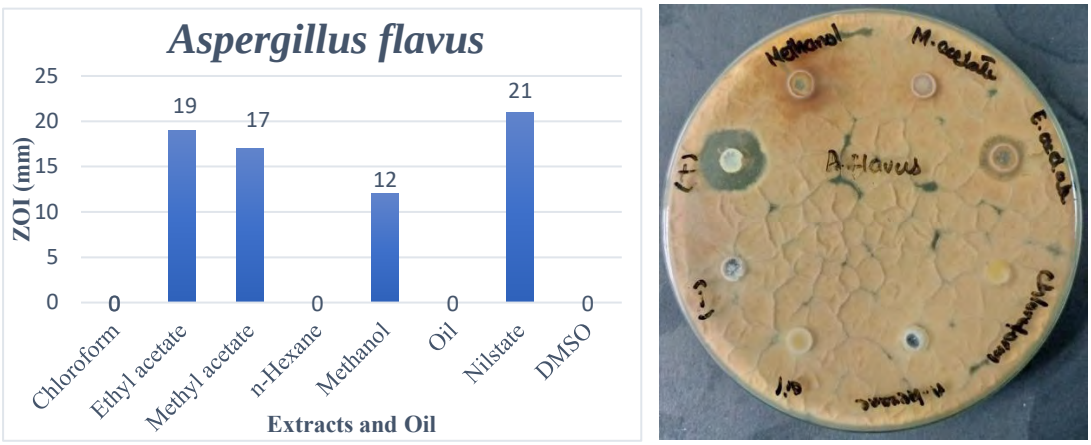


(a) **(b)**

Fig. 4.26. Antifungal activity of extracts and oil against phytopathogenic *Fusarium:* (a): Graph showing the ZOI of different extracts against *Fusarium* (b): Diagram showing the antifungal activity exhibited by *R. communis L* and different extracts against *Fusarium.*

4.11.2. Antifungal activity of extracts and seed oil against *Aspergillus flavus*

A. flavus is a crop opportunistic pathogen. It produces aflatoxin in the seeds of crops. It is carcinogenic in nature and damages maize, cottonseed, peanut, and tree nuts (Klich, 2007). Ethyl acetate showed 19mm ZOI. Methyl acetate showed 17mm ZOI respectively. Methanol demonstrated 12mm ZOI. n-Hexane, chloroform, oil, and negative control didn't show any activity while positive control showed 21mm ZOI.

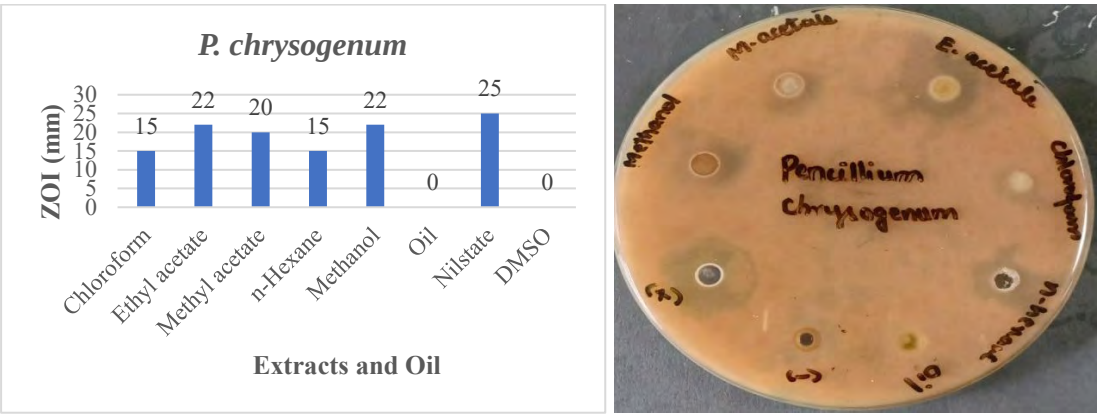


(a) **(b)**

Fig. 4.27. Antifungal activity of extracts and oil against phytopathogenic *Aspergillus flavus:* (a): Graph showing the ZOI of different extracts against *Aspergillus flavus* (b): Diagram showing the antifungal activity exhibited by *R. communis L* and different extracts against *Aspergillus flavus.*

4.11.3. Antifungal activity of extracts and seed oil against *Penicillium chrysogenum*

Penicillium species-caused blue mold is a serious fungal disease that is endangering Pakistan's viticulture sector and degrading grape quality during handling, shipping, and distribution. The key factor reducing grape shelf life, causing berry deterioration and up to 50% increase in market losses, is grapes' vulnerability to the *Penicillium* genus. This disease not only causes berries to lose weight, change color, and soften, but it also creates mycotoxins, which may be dangerous to humans. The largest genus of saprophytic fungi, *Penicillium*, has over 400 species that have been identified and are found all over the world. Pathogenic *Penicillium* species infect a variety of crops, fruits, and vegetables. *Penicillium causes* blue mold on apples, and green mold on citrus fruits. The agricultural and horticultural sectors are concerned about these fungi as they cause post-harvest fruit deterioration (Ghuffar et al., 2021). Ethyl acetate and methanol showed 22mm ZOIs. Methyl acetate demonstrated 20mm zone while chloroform and n-Hexane showed 22mm ZOIs respectively. Oil and negative control didn't show activities against it. Nilstat showed 25mm zone of inhibition.



(a) **(b)**

Fig. 4.28. Antifungal activity of extracts and oil against phytopathogenic *Penicillium chrysogenum:* (a): Graph showing the ZOI of different extracts against *Penicillium chrysogenum* (b): Diagram showing the antifungal activity exhibited by *R. communis L* and different extracts against *Penicillium chrysogenum*.

Table 4.15. Relative activity of extracts and oil against phytopathogenic fungal strains

The following table shows relative activities of castor seed extracts and oil against fungal strains.

Strains	Extracts/drug and oil							
	Methanol	**Methyl acetate**	**Ethyl acetate**	**n-Hexane**	**Chloroform**	**Oil**	**Nilstat**	**DMSO**
Fusarium	Moderate	Moderate	High	Moderate	Moderate	Moderate	High	-
P. chrysogenum	High	High	High	Moderate	Moderate	-	High	-
A. flavus	Low	Moderate	Moderate	-	-	-	High	-

4.12. MIC of *R. communis* seed extracts and oil for Phytopathogenic fungal strains

Table 4.16. MIC of *R. communis L* seed extracts and oil for phytopathogens fungal strains

Following table shows MICs values of extracts and oil against phytopathogens. Ethyl acetate, methyl acetate and methanol showed good activities.

Extracts/drug and oil	Fungal Strains		
	MIC (mg/ml)		
	Fusarium	***Penicillium chrysogenum***	***Aspergillus flavus***
Chloroform	5	50	-
Ethyl acetate	0.05	0.05	0.5
Methyl acetate	50	0.05	5
Methanol	0.5	0.05	50
n-Hexane	0.5	50	-
Oil	0.5	-	-
Nilstat	0.05	0.05	0.05

4.13. Antioxidant activity (DPPH free radical scavenging assay)

The antioxidant property of medicinal plants is connected to the DPPH scavenging of free radicals. Free radicals are the reactive oxygen species that are created in reaction to an injury or during the digestion of specific meals. Oxidative stress, which can occasionally result in cell damage or disintegration, is brought on by an excess of free radicals in the cellular environment. Antioxidants are substances that scavenge free radicals, protecting cells from harm/decay. The extracts and oil demonstrated DPPH scavenging abilities in a concentration-dependent manner (A. Haq et al., 2021). In oil, n-Hexane, methanolic, ethyl acetate, methyl acetate and chloroform extracts demonstrated noticeably stronger scavenging action.

Table 4.17. Antioxidant activity of *R. communis L* extracts and oil

Extracts and Oil	Antioxidant activity % (mg/ml)				
	500	**50**	**5**	**0.5**	**0.05**
Methanol	67	62	58	52	40
n-Hexane	52	30	22	14	12
Methyl acetate	58	54	48	40	21
Ethyl acetate	62	56	53	45	38
Chloroform	54	47	35	12	3
Oil	62	61	46	44	36
Ascorbic acid	94	93	92.9	92.4	92

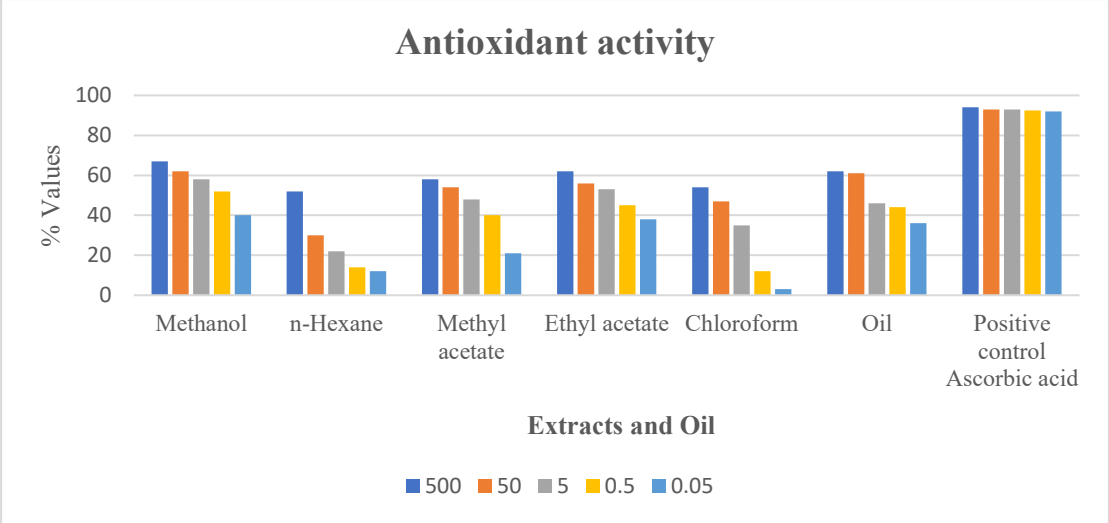


Fig. 4.29. Graph showing antioxidant activity % of *R. communis L* extracts and oil

4.14. Cytotoxic assay on Brine shrimp

Utilizing cytotoxic activity to evaluate a compound's cytotoxic action, one can employ the straightforward experiment of brine shrimp lethality. The cytotoxic experiment used *R. communis* extracts and seed oil at various concentrations. The findings showed that *R. communis* extract and seed oil's cytotoxic effects on brine shrimps were concentration dependent. Cytotoxicity of chloroform was slightly high as compared to other extracts. Ethyl acetate, methanol, methyl acetate, n-hexane and oil demonstrated no cytotoxicity. Cytotoxicity reduces with increase in polarity. Low polarity of chloroform extract can be the cause of its cytotoxicity as compared to other extracts. Chloroform can be ideal choice for tumor and cancer related studies (I. Haq et al., 2012).

Table 4.18. Cytotoxic assay of *R. communis L* extracts and oil on brine shrimps

Extracts and Oil	Dilutions (mg/ml)	Time	
		Alive cells after 24 hours	Alive cells after 48 hours
Chloroform	0.5	8	7
	0.05	9	9
Ethyl acetate	0.5	9	8
	0.05	10	9
Methyl acetate	5	10	9
	0.5	10	10
n-Hexane	0.5	10	10
	0.05	10	10
Methanol	0.5	10	8
	0.05	10	9
Oil	500	10	10
	50	10	10
Physical control		10	10
DMSO		10	10
Vincristine sulfate	0.5	0	0
	0.05	0	0

4.15. Determination of Acid value, FFA content, and Saponification value of *R. communis L* oil

For the determination of each value, specific experiments were performed in triplicates and their mean value was listed in table 4.19.

Table 4.19. Determination of physicochemical parameter of *R. communis* Oil

Experiments for each parameter were performed in triplets and an average value was calculated:

Parameter	Experimental value	Mean value
Acid value	4.48	**5.6**
	5.6	
	5.07	
FFA (%)	2.23	**2.6**
	2.81	
	2.76	
Saponification value	196.32	**210.36**
	224.4	
	210.36	
Ester value	191.84	**205.32**
	218.8	
	205.32	
% Glycerin	10.49	**11.22**
	11.96	
	11.21	

(a) **(b)**

Fig. 4.30. (a) Pink color disappeared after titration (b) blank run for saponification number

Fig. 4.31. Permanent pink after titration with KOH for FFA content

4.16. Biodiesel production from *R. communis L* oil

R. communis L oil was used to check its conversion into biodiesel through a chemical transesterification process. Initially, the reaction was conducted at standard conditions; Temperature (60ºC), Oil to Methanol ratio (1:6), Catalyst concentration (2%), RPM (600), and reaction time of 120 mins. FAME yield was 70%.

4.16.1. Optimization of Parameters for Biodiesel Production using Plackett-Burman Design

Temperature, catalyst concentration, agitation, oil to methanol ratio, and reaction time were all optimized for *R. communis L* oil biodiesel synthesis. Experiments were conducted under the parameters specified by the Plackett-Burman design, and the percentage volumetric yield was recorded.

Table 4.20. Percentage Volumetric Yield of Biodiesel in response to conditions specified by Plackett-Burman Design for each run.

The maximum volumetric yield of biodiesel i.e., 96% was recorded with run 3 with conditions: temperature 60°C, oil to methanol ratio 1:15, catalyst concentration 0.50%, RPM 900, and reaction time 60 minutes.

Runs	Catalyst Concentration %	Temperature Celsius	Oil to Methanol ratio	Agitation RPM	Reaction time minutes	% Volumetric yield of Biodiesel
1	1.50	60.00	1:9	900.00	60.00	49
2	0.50	60.00	1:9	300.00	120.00	83
3	0.50	60.00	1:15	900.00	60.00	96
4	0.50	60.00	1:15	300.00	120.00	79
5	1.50	60.00	1:15	300.00	60.00	63
6	0.50	50.00	1:9	300.00	60.00	75
7	1.50	50.00	1:15	300.00	60.00	68
8	1:00	55.00	1:12	600.00	90.00	73
9	1:00	50	1:12	300.00	90.00	55
10	1:00	60	1:15	900.00	120.00	70
11	0.50	50.00	1:15	900.00	120.00	67
12	1.50	50.00	1:15	900.00	120.00	73
13	1.50	60.00	1:9	900.00	120.00	59
14	1.50	50.00	1:9	300.00	120.00	65
15	0.50	50.00	1:9	900.00	60.00	85

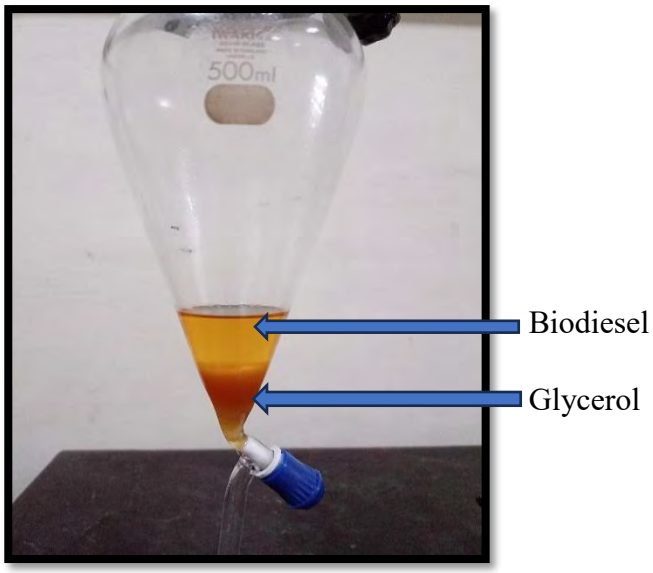


Fig. 4.32. Formation of layers of Glycerol and Biodiesel after 24 hours

4.16.2. Pareto chart

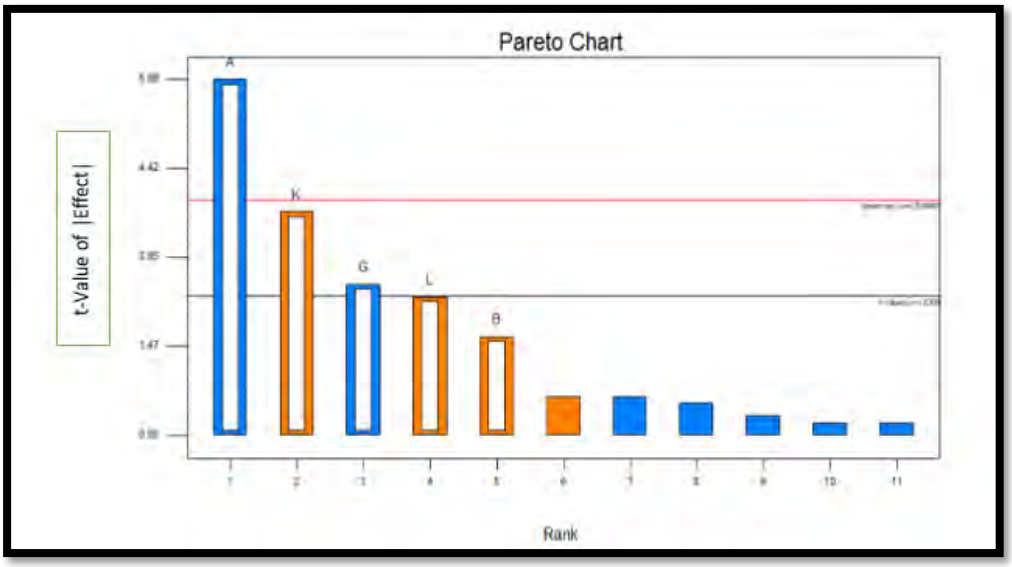


Fig. 4.33. Illustration of significant factors through Pareto Chart

This pareto chart represents number of significant factors. Blue represents a negative influence, whereas orange shows a favourable effect on biodiesel output.

A: Catalyst concentration

B: Oil to methanol ratio

K: Temperature

L: Agitation

G: Reaction time

Catalyst concentration (A), temperature (K), reaction time (G) are 3 significant factors which have have positive effect on Biodiesel yield. Oil to methanolratio (B), and agitation (L) are non-significant factors negative effect on biodiesel yield

4.16.3. ANOVA for selected factorial model

4.16.3.1. Analysis of various table [Partial sum of squares- Type lll]

	Sum of Squares	df	Mean Square	F Value	p-value prob>F	
Source Model	1755.67	5	351.13	12.54	0.0013	Significant

The Model F-value of 12.54 suggests the model is significant. There is only a 0.13% chance that a “Model F-Value” this large might happen owing to noise only.

When “Probe>F” is less than 0.0500, model terms are considered significant. In this instance A, G, K are significant model terms. If the values are higher than 0.1000, indicate the model terms are non-significant. Model reduction may enhance your model If it has many unnecessary model terms (excluding those necessary to maintain hierarchy).

The "Curvature F- value" of 2.92 indicates that the curvature in the design space (as determined by the difference between the average of the center points and the average of the factorial points) is not significant in comparison to the noise. A big "Curvature F-value" is indicative of noise, which has a 12.61% chance of occurring.

According to the "Lack of Fit F-value" of 0.07, the lack of fit is not significant in comparison to the pure error. A "Lack of Fit F-value" this large could be caused by noise with a 99.51% probability. A minor fit difference is advantageous. The model must fit our data.

Std. Dev.	5.29	**R-Squared**	0.8868
Mean	70.67	**Adj R-SQUARED**	0.8161
C.V.%	7.49	**Pred R-Squared**	0.7232
PRESS	570.50	**Adeq Precision**	13.555

The "Pred R-Squared" of 0.7232 and the "Adj R-Squared" of 0.8161 are reasonably consistent. The signal-to-noise ratio is calculated by "Adeq Precision." A ratio of at least 4 is preferred. The signal is adequate, as evidenced by our ratio of 13.555. To navigate the design space, utilize this model.

4.16.3.2. Final Equation in terms of code factors

Volumetric Biodiesel Yield= +71.83 -9.00 *A +2.50 *B -3.83 *G +5.67 *K + 3.50 *L

4.16.3.3. Final Equation in terms of Actual Factors:

Volumetric Biodiesel Yield = +79.83333 -18.00000*Catalyst concentration+0.83333* Oil to methanol ratio-3.83333 *G+5.66667 *K+3.50000 *L

4.16.4. Plots of volumetric yield of biodiesel versus factors

4.16.4.1. Plot of volumetric yield (Response) versus catalyst concentration (Significant factor)

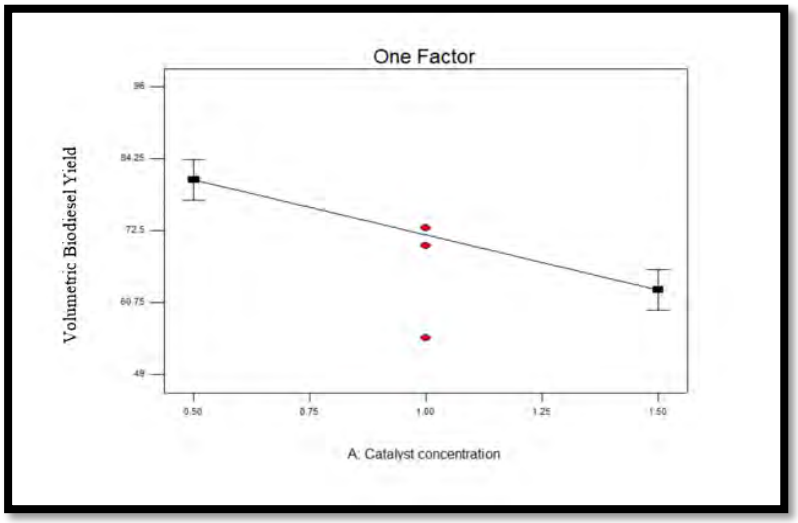


Fig. 4.34. Plot of volumetric yield (Response) versus catalyst concentration (Significant factor)

This graph states that catalyst concentration has a negative effect on the volumetric yield of biodiesel. By decreasing the concentration of catalyst, we can subsequently increase the biodiesel production from *R. communis* oil.

4.16.4.2. Plot of volumetric yield versus Oil to Methanol ratio (non-Significant factor)

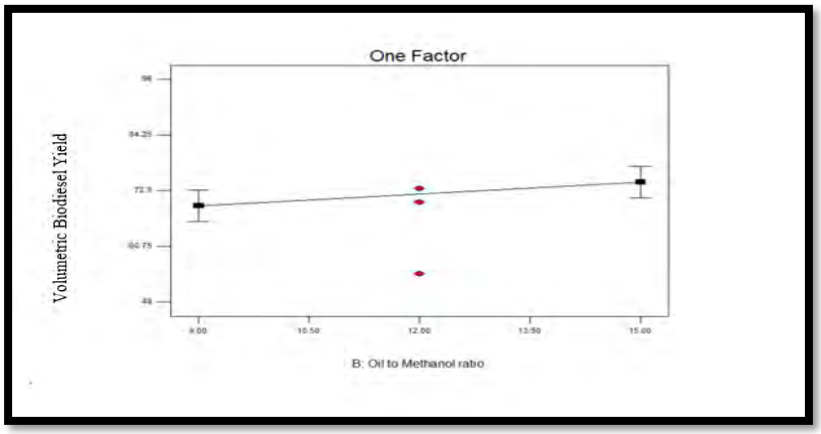


Fig. 4.35. Plot of volumetric yield versus Oil to Methanol ratio (non-Significant factor)

This graph states that the oil to Methanol ratio has a non-significant effect on the volumetric yield of biodiesel. By increasing the oil to methanol ratio, there is a small increase in biodiesel yield from *R. communis* oil.

4.16.4.3. Plot of volumetric yield versus Temperature (Significant factor)

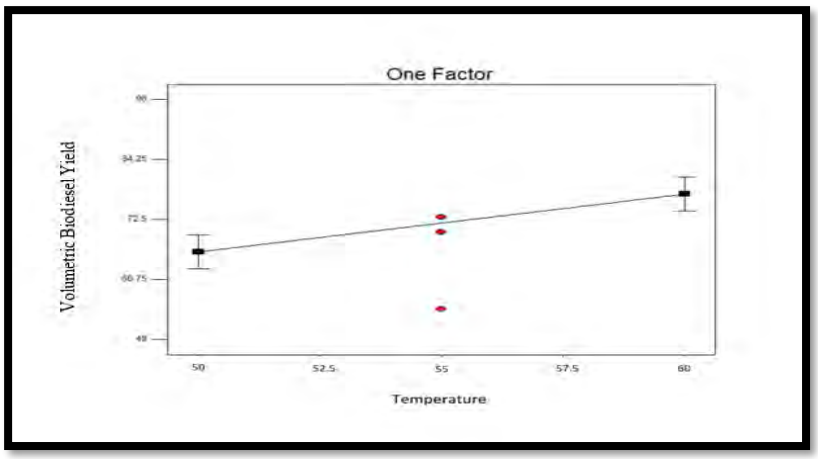


Fig. 4.36. Plot of volumetric yield versus Temperature (Significant factor)

This graph states that temperature has a positive effect on the volumetric yield of biodiesel. By increasing the temperature, we can subsequently increase the biodiesel production from *R. communis* oil.

4.16.4.4. Plot of volumetric yield versus Reaction time (Significant factor)

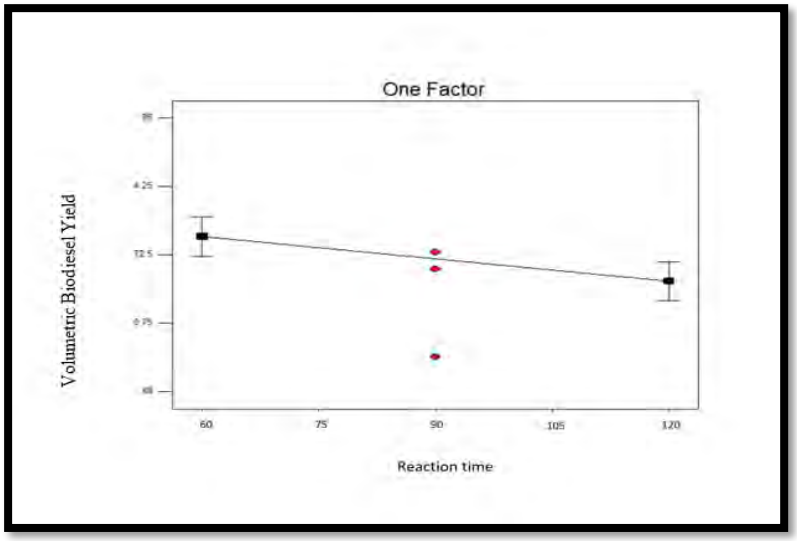


Fig. 4.37. Plot of volumetric yield versus Reaction time (Significant factor)

This graph states that Reaction time has a negative effect on the volumetric yield of biodiesel. By decreasing the reaction time, we can subsequently increase the biodiesel production from *R. communis* oil.

4.16.4.5. Plot of volumetric yield versus Agitation (non-Significant factor)

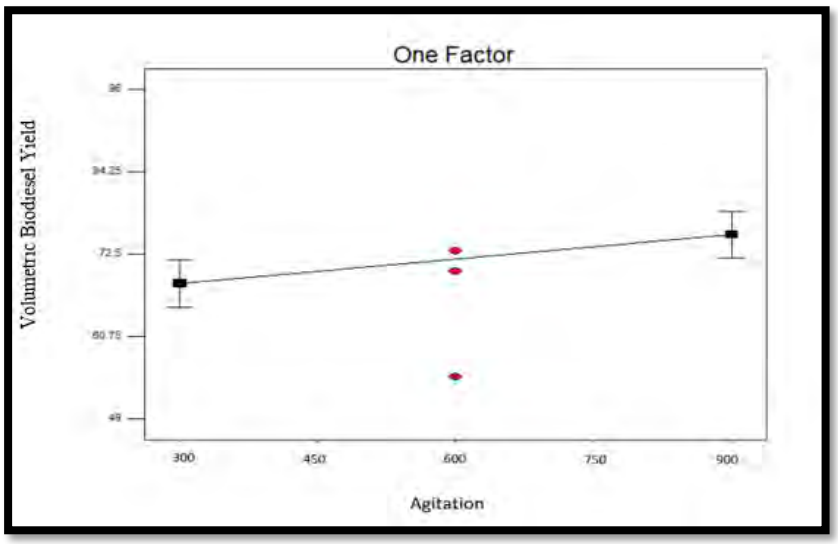


Fig. 4.38. Plot of volumetric yield versus Agitation (non-Significant factor)

This graph states that agitation has a non-significant effect on the volumetric yield of biodiesel. By increasing the agitation, biodiesel production increases a little bit from *R. communis* oil.

4.17 FTIR analysis of FAME produced by alkali catalyzed trans-esterification of *R. communis L* oil

FTIR analysis of the biodiesel was performed to confirm the FAME presence in the reaction mixture. FTIR spectra of FAME of *R. communis* was obtained at conditions of 1% KOH catalyst, 1:12 oil to methanol ratio, at 60°C, 600 rpm stirring, and 120 mints reaction time. One major and two minor stretches of ester bands were observed at 1741.16, 1198.16, and 1035.34 confirming the presence of FAME in the reaction content. Peaks at 1198.16 and 1172.19 correspond to the C-O stretches for esters and these stretches are only present in the FAME confirming the biodiesel production from castor oil.

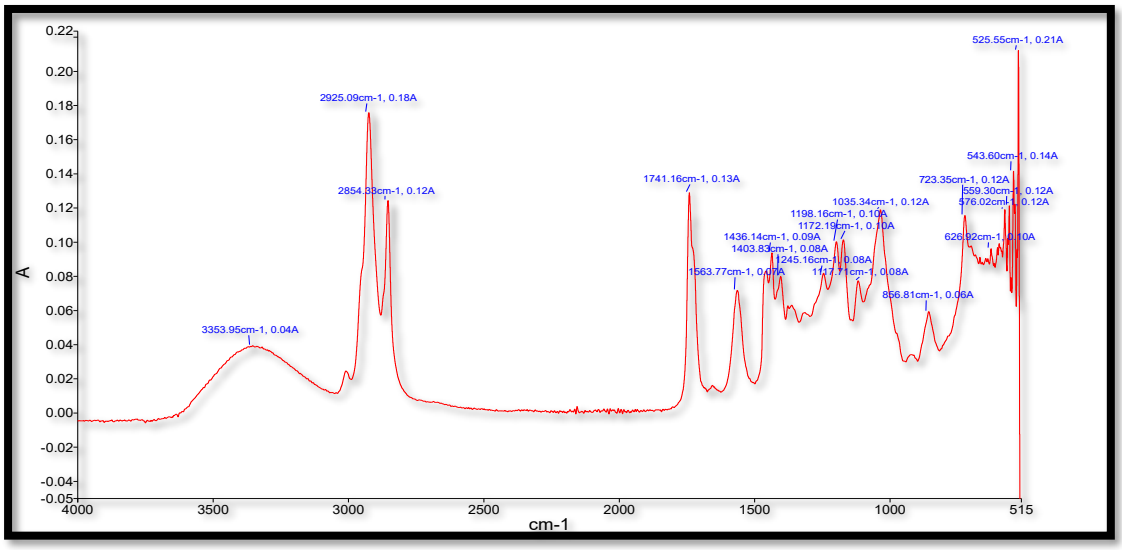


Fig. 4.39. FTIR spectra of FAME produced by alkaline trans-esterification of *R. communis L* oil.

Chapter # 5
Discussion

Discussion

Health, environment, and energy are necessary for survival of humans on earth. Modern living standards have created threats to ecology. Nowadays the world is facing serious issues on energy crisis and antimicrobial resistance. So, there is a need for new sources to overcome these issues. Plant based material is the best option for energy and pharmaceutical compounds production. Diesel made from fossil fuels can be replaced with castor biodiesel because it is sustainable and renewable. It has lower greenhouse gas emissions and is made from castor seed oil.

Castor seeds contain a variety of phytochemicals, including ricinoleic acid, tanins, alkaloids, phenols, saponins, steroids, resins, glycosides etc. which have anti-inflammatory and antibacterial activities. Castor seeds are a rich source of natural compounds because of these phytochemicals' potential uses in healthcare, cosmetics, and other industries as reported in study (Abel et al., 2022).

R. communis L oil has the potential for biodiesel production. In the current study, castor seed had 20% oil content. It is related to the value given by (Momoh et al., 2012) that castor has 20% volatile oil. Numerous factors, including seed ripening and plant watering, have an impact on the oil content. *R. communis L* oil had 2.6% FFA level that is according to (Panhwar et al., 2016) acid value of 5.6mg /g of oil was in range according to literature (Bale et al., 2022), saponification value 210.36 mg of KOH/g of oil that in range described by (Ferdous et al., 2013). It has ester and glycerin values 205.32mg/g and 11.22 % respectively. Therefore alkali-based trans-esterification was carried out. On an alkali basis, the trans-esterification of *R. communis L* oil depends on the molar ratio, catalyst concentration, reaction time, agitation, and reaction temperature. FTIR analysis has confirmed the FAME peaks at 1741.16, 1198.16, 1035.34 which are different from oil's FTIR analysis. Peaks in oil (1742.83,1162.52 and 1097) were transformed into biodiesel. These FAME peaks ranges were reported in previous study (A. Haq et al., 2020). The maximum volumetric yield of biodiesel was recorded 96%, with conditions: temperature 60°C, oil to methanol ratio 1:15, catalyst concentration 0.50%, RPM 900, and reaction time 60 minutes. In previous study (Keera, Sabagh, et al., 2018) at 0.5% NaOH catalyst, 70°C, 3:1 ethanol: oil, and 3 h reaction resulted in a conversion of 96.2% biodiesel yield. The molar ratio affects

the yield of FAME. Maximum yield was obtained at 1:15 oil to methanol ratio. A higher ratio of methanol makes the reaction efficient and produces more yield. In previous study at 60^{o}C and 1% catalyst biodiesel yield was increased from 65% to 95% by increasing the molar ratio from 1:6 to 1:9 (Keera, El Sabagh, et al., 2018). Catalyst concentration is an important factor for the optimization of biodiesel production. In the current study 0.5-1.5% catalyst was used. Maximum yield was obtained at 0.5 %. Increased in catalyst concentration didn't increase FAME production because extra amount of catalyst reacts with the triglycerides and free fatty acids of the oil and produce emulsion. It increases the viscosity that produce viscous gel as a result separation of glycerol layer and yield of ester lost (A. Haq et al., 2023). In previous study when catalyst concentration was increased from 1% biodiesel yield was decrease from 95% due to emulsion formation (Keera, Sabagh, et al., 2018) (Ramezani et al., 2010). In this study maximum yield of biodiesel was obtained at 60^{o}C. Different reactions were performed at 50^{o}C, 55^{o}C and 60^{o}C. FAME yield increased by increasing the temperature. Temperature above then 60°C (boiling point of methanol) was ignored due to fast reaction of saponification than the transesterification reaction. Temperature above the boiling point causes excessive loss of methanol which causes incomplete reaction and loss of biodiesel yield. In (Keera, Sabagh, et al., 2018) study at 60^{o}C and 30^{o}C, 87% and 90% yield was obtained respectively. Agitation rate was selected 300-900rmp along with other parameters. Maximum yield was obtained at 900rpm. Castor oil transesterification responses depend non-significantly on the agitation rate. It affects heat dispersion, mass transfer, reaction kinetics, and reaction efficiency in general. The optimal agitation rate can change depending on the reactor design, catalyst type, reactant concentrations, and desired reaction conditions, among other things. It's crucial to strike a balance between effective mixing and preventing excessive emulsification or other negative consequences that can impede the reaction (Ramezani et al., 2010). Maximum yield of biodiesel was obtained at 60 minutes. Reaction time is also an important factor for optimization of biodiesel because biodiesel production is quick throughout the transesterification reaction's initial phases until the reaction achieves equilibrium. After reaching the ideal point, the reaction begins to reverse in the opposite direction, towards the reactants. The reversibility of the transesterification reaction is

what caused this anomaly. When reactant is lacking, catalyst tends to adsorb products. Because the catalyst (CaO) can absorb the product, a long reaction time also lowers the yield of biodiesel. Determining the ideal transesterification reaction response time is so crucial (Ismail et al., 2016). In (Keera, Sabagh, et al., 2018) study after 60 minutes biodiesel yield become constant. Current study demonstrates that castor oil has great potential to be used to produce biodiesel.

In today's modern world, the discovery of antibiotics was seen as the beginning of a scientific revolution. During World War II, the antibiotic Penicillin rescued millions of lives. Antibiotics were believed to potentially save lives before the development of antimicrobial resistance. Antimicrobial medications, which could once be used to destroy microbes quickly, are no longer effective. To achieve the Sustainable Development Goals (SDGs), rapid action is required. AMR is one of the top ten global public health concerns now. Even though AMR is believed to be a natural phenomenon, misuse and abuse of these antimicrobial drugs contribute to the emergence of resistance. For a very long time, various plants, and their components, including flowers, buds, leaves, stems, seeds, skin, and pulp have been used to enhance the flavor and aroma of food. Plants contain a wide variety of secondary metabolites as well. The seed has rubefacient and stimulating properties (Aminov, 2010) (Lobanovska & Pilla, 2017).

Nature has not only provided us with food but also with means to heal our sicknesses. Therefore, new efforts are being undertaken to replace the current antibiotics with natural resources including castor plant seeds, traditional methods, and herbal treatments (Anand et al., 2019).

Researchers are most intrigued by *R. communis L* because of its abundance of medicinal benefits, reasonable cost, and potential for bioactivity. *R. communis L* has enormous untapped potential for use in medicine. *R. communis L* has been the subject of more inquiry recently as scientists try to understand its true potential (Yeboah et al., 2020). Like this, *R. communis L* can be grown in certain areas of Pakistan due to their favorable climate and environment.

According to research on the phytochemical makeup of *R. communis L* seeds and their chemical makeup, the seeds included a wide range of compounds. Current study has

confirmed that castor seed extract and oil contain vital secondary metabolites such as alkaloids, flavonoids, phenols, alkaloids, resins, glycosides, saponins, and tannins, that are frequently found in conventional medicines and utilized as therapies for a few illnesses. Similar results have been observed in the literature (Anand et al., 2019). FTIR analysis has also confirmed different functional groups are present in extracts and oil. Extracts and oil have alkenes, alkanes, aromatic, alcoholic compounds. They have alkyl, ester, ether, carbonyl, amines, methyl, methylene, carboxylic ketone, aldehyde, functional groups. Carbohydrates are also present in castor seed extracts and oil as reported in previous study (Panhwar et al., 2019).

The current study examined the antimicrobial activity of *R. communis L* in treating diseases brought on by bacterial and fungal strains which are a growing concern among hospitalized patients. *S. aureus*, a gram-positive bacterium, infects hospital patients severely and is the source of diseases like pneumonia and meningitis (Momoh et al., 2012). *E. coli, P. aeruginosa, K. pneumoniae, S. enterica, and S. epidermidis* are all capable of causing blood infections such as urinary tract infections (UTI), respiratory tract infections, dysentery-like diarrhea, wounds, burns, and other blood infections. ATCC (*E. coli, P. aeruginosa, K. pneumoniae, S. enterica, B. subtilis, S. aureus, Enterobacter, S. epidermidis)* and MDR strains (*Salmonella, K. pneumoniae, P. aeruginosa*) were used to find out the antimicrobial potential of castor extracts and oil. *Candida albicans, Aspergillus flavus, Aspergillus niger, Fusarium oxysporum,* and *Curvularia lunata* were human pathogens that cause severe infections and diseases and phytopathogens *Aspergillus flavus, Fusarium* and *P. chrysogenum* were selected. The current study looked at the fact that castor oil has less activity than its extracts, even though castor seed extracts have the highest activity against tested microbes and can be utilized for the treatment of bacterial and fungal infections.

Methanol, methyl acetate, ethyl acetate, chloroform, and n-Hexane solvent extracts showed good activity against bacterial ATTC and fungal strains while methanol, methyl acetate, ethyl acetate, chloroform showed high to low activities against MDR bacterial strains. Ethyl acetate extract showed high activities against bacterial strains while against fungal strains it showed a high to moderate range of activities. For MDR strains it showed high activities. Ethyl acetate showed more activities against *E. coli* (28mm)

S. aureus (30mm), *K. pneumoniae* (32mm), *P. aeruginosa* (27mm), and *C. albicans* (16mm) as compared to reported values of castor leaves (Kebede & Shibeshi, 2022). Methanolic extract showed high activity against bacterial species but showed high to low-range activities for fungal strains in current study. Methanolic extract showed 23mm zone inhibition against *B. subtilis* and 21mm against *E. coli* which are greater than described in literature that were 8.3 mm against *B. subtilis* and showed no ZOI against *E. coli* (Javaid et al., 2015). Seed cake extracts showed greater activity against *S. aureus* (29mm ZOI) than leaves methanolic extracts that is present in previous study (Naz & Bano, 2012). Methanolic extract showed higher ZOI against *A. flavus* that was 20mm than the literature while methanolic extracts showed 14mm ZOI against *A. niger* which was less than mentioned by (Javaid et al., 2015). Methanolic extract showed high activities against strains *S. aureus*, *E. coli*, *K. pneumoniae* and *P. aeruginosa* as compared to previous study (Voleti et al., 2022) (Kebede & Shibeshi, 2022). Methanolic extract showed 20mm,18mm, and 20mm ZOI against *P. aeruginosa, K. pneumoniae and Salmonella* MDR strains respectively. Methyl acetate showed a high to moderate activity range against bacterial strains while against fungal strains high to low range. It showed good ZOI for *P. aeruginosa, K. pneumoniae and Salmonella* MDR strains. Chloroform showed 13mm ZOI against *E. coli* which is a little bit less than mentioned in literature that had 14mm ZOI. Chloroform showed 17mm ZOI against *B. subtilis* that is better than described by (Javaid et al., 2015). It showed 9mm ZOI against *K. pneumoniae* MDR strain. Chloroform showed greater activity for *A. niger* with 20mm ZOI while against *A. flavus* it showed 14mm zones which was a little bit less than described by (Javaid et al., 2015). Chloroform showed high to low range values for both types of strains. n-Hexane showed a high to low range of values against bacterial species while it didn't show any activity against MDR strains. It showed high activities against *A. niger* and *C. lunata* but didn't show any activities against other strains. In previous study (Kebede & Shibeshi, 2022) n-Hexane extract didn't show any activity against *P. aeruginosa* but in current study it showed low activity. In our study oil only showed activity against ATCC *E. coli* and phytopathogenic *Fusarium.* It showed greater activity against *E. coli* than reported value (Momoh et al., 2012). Cator oil showed 19mm ZOI against phytopathogenic *Fusarium.* The (Tishreen University & Janoud, 2017) study showed that oil has inhibitory activities for *Fusarium.* Previous

study (Mahilrajan et al., 2014) demonstrated that castor oil don't have significant activities against phytopathogenic *A. flavus*, and *Penicillium* spp. In current study oil didn't show activities against *P. chrysogenum* and *A. flavus*. Bacterial and fungal species may have shown resistance to active compounds of oil due to the production of different enzymes, spores, and toxins which have produced resistance against oil components (Momoh et al., 2012). Tetracycline and nilstat were used as positive controls against bacterial and fungal strains. Tetracycline showed high activities for ATCC stains but for MDR strains it showed high to moderate activities. Nilstat showed high activities against fungal strains. DMSO (as negative control) didn't show any activities against both types of pathogens as reported by (Hajrah et al., 2018).

MIC for bacterial and fungal strains, and antioxidant activity of extract were calculated according to the procedure mentioned (Iqbal et al., 2012). Dilutions with concentrations 500mg/ml, 50mg/ml, 5mg/ml, 0.5mg/ml and 0.05mg/ml were prepared. Ethyl acetate showed 0.05mg/ml MIC value against all ATCC bacterial and *Salmonella* MDR strains while for MDR *K. pneumoniae* and *P. aeruginosa* it showed 0.5mg/ml MIC value. For *F. oxysporum, C. albicans,* and *A. flavus* (clinical and phytopathogen) its MIC value was 0.5mg/ml. 5mg/ml value was obtained for *A. niger* and *C. lunata* and 0.05mg/ml MICs values for phytopathogens *P. chrysogenum* and *Fusarium*. In literature ethyl acetate and chloroform extracts showed 0.156 µl/ mg MIC value against for *E. coli* and *S. aureus*, 0.313 µl/ mg for *B. subtilis.* n-Hexane showed 0.313 µl/ mg, 0.156 µl/ mg and 1.25 µl/ mg MIC vales for these strains respectively (Iqbal et al., 2012). In this study n-Hexane showed a 500-0.5mg/ml range of MICs values for ATCC and MDR bacterial stains and for fungal strains it showed low range of values. It demonstrated 0.5mg/ml and 50mg/ml for *Fusarium* and *P. chrysogenum* respectively. Oil only showed 500mg/ml MIC value for *E. coli* and 0.5mg/ml MIC for *Fusarium.* According to (Mahilrajan et al., 2014) study castor oil didn't show good MIC values for *A. flavus* and *Penicillium.*

MIC values of methanolic extracts against *S. aureus*, *E.coli*, *K. pneumoniae* were reported as 6.25mg/ml-25ml/ml (Kebede & Shibeshi, 2022). Methyl acetate showed 0.5mg/ml values for all ATCC strains. For MDR stains *Salmonella* (5mg/ml)*, K. pneumoniae* (50mg/ml) and *P. aeruginosa* (0.5mg/ml) it showed good MIC values. Methyl acetate showed 0.5mg/ml MIC values for all clinical fungal strains expect *A.*

niger against it showed 5mg/ml value. For phytopathogenic *A. flavus, P. chrysogenum and Fusarium* it showed 5mg/ml, 0.05mg/ml and 50mg/ml values of MICs. Methanolic extract showed 0.5mg/ml values for all ATCC and MDR bacterial strains except ATCC *S. aureus* against it showed 0.05mg/ml MIC value. For fungal strains it showed a 50-0.05mg/ml range of MIC values. In previous study methanolic extract showed 50mg/ml MIC value for *C. albicans* (Kebede & Shibeshi, 2022). Chloroform extract showed 50-0.5mg/ml range for ATCC bacterial strains, 50mg/ml for MDR *K. pneumonia* and 50-0.05mg/ml range of MIC values for selected fungal strains. In previous study (Iqbal et al., 2012) chloroform showed 0.156 µg/ml MIC value for *E. coli* and *S. aureus* and for *B. subtilis* 0.313µl/ml value. In previous study (Mdee et al., 2009) acetone castor seed extract demonstrated anti phytopathogenic activity. It showed 0.84 mg/ml MIC for *F. oxysporum,* 0.64mg/ml for *A. parasiticus* and *A. niger* and 0.43mg/ml for *P. expansum* while in current study methanol, methyl acetate and ethyl acetate showed 50mg/ml, 5mg/ml, and 0.5 mg/ml MICs against *A. flavus* respectively. Methanol, n-Hexane, and oil showed 0.5mg/ml value while methyl acetate, ethyl acetate and chloroform showed 50mg/ml ,0.05mg/ml and 5mg/ml MICs respectively against phytopathogenic *Fusarium* fungal strain. MIC value of methanol, methyl acetate, and ethyl acetate was 0.05mg/ml for *P. chrysogenum* while chloroform and n-Hexane showed 50mg/ml against it. Ethyl acetate, methanol, n-Hexane, and oil showed good activities as compared to acetone for *Fusarium spp.* Ethyl acetate was a good solvent for *Aspergillus spp.* as compared to acetone. Methanol, methyl acetate and ethyl acetate can be excellent solvents of choice for *P. chrysogenum* as compared to acetone. Results of current study support the antimicrobial potential of castor seeds.

Cator seed extracts and oil have good antioxidant activity. 500mg/ml of oil and each extract showed maximum antioxidant activity. Methanol showed the highest activity (67- 40%). n-Hexane showed 52-12% antioxidant activity. Methyl acetate, ethyl acetate and chloroform showed 58-21%, 62-38 % and 54-3% activities. Castor oil demonstrated 62-36% activity. Castor seeds have good antioxidant activities. Methanol, ethyl acetate, n-Hexane and chloroform showed high results. In (Abbas et al., 2018) study different extraction methods, Shaking , sonication and Soxhlet extraction were used to prepare methanolic extracts of seeds and then antioxidant activity was evaluated. They gave 7.25%, 8.8% and 7.42 % for 0.1mg/ml methanolic extracts. In

previous study, methanol, ethyl acetate, n-Hexane and chloroform extracts prepared from aerial parts of castor showed 26, 41, 12, and 41% activities respectively (Ahmed, 2018).

Cytotoxic activity of different seed extracts and oil on brine shrimps showed that chloroform has slight cytotoxic effect. It might be due to the low polarity of chloroform. Other extracts and oil can be utilized for inhibition of microbes and as an antioxidant (I. Haq et al., 2012). In previous study (Abbas et al., 2018) methanolic extract of seed demonstrated cytotoxic effect while in our study it didn't show it. Results of cytotoxic assay confirmed that ethyl acetate, methyl acetate, methanol, n-hexane solvents, and oil can be good choices for drug preparation.

The results of the current study demonstrate that *R. communis L* seed extracts have strong antimicrobial properties. Its legitimacy as a potential competitor in the pharmaceutical industry is increased by the presence of major bioactive compounds with antibacterial and antifungal properties. To find bioactive compounds that could be used as potent antimicrobials for therapeutic purposes as an alternative drug, more research is needed.

The results of the current study demonstrate that the *R. communis L* plant is an excellent feedstock for biorefineries. The study's findings support the notion that *R. communis L* seed oil can be used to chemically trans-esterify to produce biodiesel. It is possible to produce bioactive chemicals using *R. communis L* extract.

Conclusion

Conclusion

The world is facing different issues related to the energy crisis and antibiotic resistance. Fossil fuels are running out more quickly. To deal with energy requirements for daily use there is a need for new sustainable, renewable, and cheap sources. For this purpose, many countries are making biofuels from biomass. For countries like Pakistan, it's better to obtain energy from cheap sources. *R. communis L* is a feedstock for biodiesel production. In the current study *R. communis L* seed oil was used to chemically trans esterify oil to produce biodiesel. The highest volumetric yield of biodiesel 96% was recorded, with conditions: temperature 60°C, oil to methanol ratio 1:15, catalyst concentration 0.50%, RPM 900, and reaction time 60 minutes.

Nature has always provided us with cures for diseases. The most modern and complex medications ought to come from natural sources. Emerging global health concerns include antibiotic side effects and drug-resistant infections. The current study also helped with the investigation of the antimicrobial properties of seed oil and extracts from *R. communis L*. The crude extracts were found to have good antimicrobial activities against all selected strains. *R. communis L* oil only showed low activity against *E. coli*. Ethyl acetate, methyl acetate, methanolic, n-Hexane and chloroform showed a good range of MICs values. Maximum antioxidant percentage was showed by 500mg/ml concentration of all extracts and oil. Extracts and oil have no cytotoxicity on brine shrimps. The results of the current study demonstrate that the *R. communis L* plant is an excellent feedstock for biorefineries.

Future Prospects

Future prospects

1. After further purification using a variety of methods, the crude phytochemicals isolated from *R. communis L* seeds can be employed individually or in conjunction with other antibiotics to fight a variety of pathogenic strains.
2. After being purified from *R. communis L* extracts, bioactive chemicals can be employed for enzyme inhibition and in vivo tests.
3. Along with chemical trans-esterification, biological transesterification of *R. communis L* can also be performed either by using lipase enzyme or whole cell approach.
4. Biogas can also be produced by using the residues left after the extraction process and their effect can be checked on the stages of the anaerobic digestion.
5. It is possible to study how various pretreatments, such as ionic solvents, alkaline, and enzymatic pretreatments, affect seed cake residues (seed cake after extraction).

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Evaluation of the Potential of Ricinus communis L (Castor) Seed as a Substrate for Biorefinery Applications

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