STUDY ON EXTRACTION, ISOLATION, CHARACTERIZATION, AND BIOLOGICAL ACTIVITIES OF A PURE COMPOUND FROM *ENTADA RHEEDEI*

Mamata Kawade[1], Varsharani Ingole[2], Siraj Shaikh[3], Sushma Katade[4]*

[1,2,4*] Department of Chemistry, PES's Modern College of Arts, Science & Commerce, (Savitribai Phule Pune University) Ganeshkhind, Pune-411016, India

[1]Department of Engineering Sciences, Ajeenkya D Y Patil School of Engineering, (Savitribai Phule Pune University), Pune-412105, India

[2]Department of Chemistry, STES's Sinhgad College of Science, (Savitribai Phule Pune University) Pune-411041, India

[3]Department of Chemistry, M.C.E. Society's Abeda Inamdar Senior College of Arts, Science and Commerce (Autonomous), Pune-411001, India

Email: sushmarkatade@gmail.com[4*]

ABSTRACT

The present study focuses on the extraction, isolation, characterization, and evaluation of the biological activity of a pure compound from *Entada rheedei*, a plant known for its traditional medicinal uses. Seed kernels of the plant were collected, authenticated, and subjected to Soxhlet extraction using polar (methanol), semi-polar (ethyl acetate), and non-polar (pet ether) solvents. The yields obtained from the ethyl acetate and pet ether extracts were 7.64 g and 5.2 g, respectively. Pure compound isolation was achieved using column chromatography, yielding 0.40 g and 2.8 g from the respective extracts. The compound was identified as a triglyceride ($C_{71}H_{126}O_6$) through spectroscopic techniques such as *^{1}H NMR*, *^{13}C NMR*, *FTIR*, *LC-MS*, and *2D NMR*. The antimicrobial effects were evaluated against various bacterial and fungal strains using agar diffusion method. The purified molecule had modest antibacterial activity, with inhibition zones of 12 mm to 17 mm and similar to conventional antibiotics. Multiple organisms (*E. coli*, *Aspergillus niger*, and *S. aureus*) have MIC values of 0.5 µl/mL. The chemical was further tested for action against tumour cell lines using the MTT assay and antioxidant activity using the DPPH assay. Both anticancer and antioxidant results support therapeutic uses for the bioactive impact potential. The data suggest that bioactive compound, isolated from *Entada rheedei*, warrants additional follow-up studies for optimization and medicinal formulation considerations.

Keywords: Entada rheedei, extraction, isolation, characterization, triglyceride, antimicrobial activity, minimum inhibitory concentration (MIC), 1H NMR, 13C NMR, biological activity.

1. INTRODUCTION

Entada rheedei, known as gigantic wetland bean, is a tropical leguminous species that occurs in Africa, India, and Southeast Asia. *Entada rheedei* is a member of the *Fabaceae* family[1]. It is widely known for its valuable pods which consist of numerous bioactive seed makeup. The bioactive compounds of *Entada rheedei*, which exhibit pharmacological properties that includes anti-inflammatory, antimicrobial, antioxidant, antidiabetic, and anticancer activity have aroused research interest. The traditional management use and practice by indigenous medicine of *Entada rheedei* for managing the treatment of several conditions spanning from asthma to wounds, and to familial gastrointestinal tract related issues among others rely on the bioactive contents of *Entada rheedei*, including, alkaloids, flavonoids, saponins, and tannins[2]. The motivation for finding new bioactive compounds to develop into pharmacological agents is driving a focus on isolating and characterizing pure compounds from *Entada rheedei*[3]. It is also worth mentioning the chemical nature of the compounds when discussing the solvents that are used for extraction. To generally look at the biological activities, and to practice the investigation, characterization, isolation, and purity extraction of *Entada rheedei* would be a valuable opportunity for creating potential pharmacological agents and other therapeutic uses[4]. Deriving active materials from plant materials is normally done using various methods of extraction with solvents (for example, maceration, Soxhlet extraction, and cold extraction)[5]. Both the solvent utilized and the part of the plant used for extraction, such as leaves, seeds, or roots, and the extraction methods will certainly affect the extraction. Hexane, chloroform, methanol, and ethanol, are common extraction solvents used to extract phytochemicals from *Entada rheedei*. Once the extraction is performed, bioactive compounds are isolated and purified using various chromatographic methods, for example, thin layer

chromatography (TLC), high performance liquid chromatography (HPLC), and column chromatography[6]. Isolation techniques separate distinct substances based on chemical features (such as size, solubility, and polarity). Of the multiple methods to isolate a compound, preparative HPLC may be the best method to isolate and purify a single compound, while at the same time yielding an efficient and producing a good amount. Advanced spectroscopic approaches, such as nuclear magnetic resonance (NMR), mass spectrometry (MS), and infrared (IR) spectroscopy, are often employed to characterise isolated compounds. These methods can be helpful for either the identification of functional groups, determining the purity of the isolated compound, or finally elucidating the structure of the compounds extracted from this bioactive entity [7]. The discovery of novel therapeutic agents may be facilitated by the purification of isolated compounds from *Entada rheedei* extracts, which will further our understanding of its chemical components. The structural determination and comprehension of the mechanisms of action of bioactive compounds from *Entada rheedei* are contingent upon this characterization [8]. The isolated and purified compound can be further elucidated and its structural details can be provided by a variety of advanced techniques. The use of protons (1H) and carbon (13C) Nuclear Magnetic Resonance (NMR) is a popular technique for finding functional groups and understanding the interactions between atoms in a molecule. Mass spectrometry (MS) is utilized to verify the bioactive compound structure and provides the molecular weight as well as the fragmentation pattern of the agent [9]. The infrared (IR) spectroscopy method also informs the functional groups as it measures absorption of the sample across a spectrum of wavelengths.

The isolated compound can be provided with a highly detailed three-dimensional structure. A technique that entails determining the arrangement of atoms in a crystalline sample [10]. Researchers are able to determine and analyze the electronic structure along with any absorption properties in the ultraviolet-visible range using UV-V is spectroscopy, which is especially helpful for compounds with conjugated systems. The characterization process also includes the comparison of the spectral data of the isolated compound with known reference materials in order to confirm its identity as well as to assess its purity[11]. The findings from such characterization methods are critical in understanding the compound's chemical behavior and possible biological effects. Research is primarily focused on the biological activities of purified compounds isolated from *Entada rheedei* since many of these compounds possess a wide range of therapeutic properties. Studies related to compounds derived from *Entada rheedei* have examined these compounds extensively for their antimicrobial activity, which has been effective against organisms such as bacteria and fungi [12]. Plant materials' antibacterial activities are most likely attributable to alkaloids, flavonoids, and saponins, which may undermine the integrity of microbial cell membranes and cause death. Furthermore, numerous research has been conducted on Entrance rheedei components, particularly their antioxidant properties, since oxidative stress is a prominent molecular route in many chronic illnesses, including cancer and cardiovascular disease. *Entada rheedei* flavonoids and polyphenolic components have also been proven to have advantageous free radical scavenging activities, making them an important natural antioxidant source.

Studies have also explored the antidiabetic properties of compounds from *Entada rheedei*, with reports showing that bioactive compounds reduce blood glucose levels and insulin sensitivity and reduce a number of other complications of diabetes. Studies have also indicated that *Entada rheedei* has substantial anti-inflammatory activity which may decrease the symptoms of inflammatory bowel disease, arthritis, neurodegenerative diseases, and other inflammatory diseases[13]. Some isolated compounds from the plant have been shown to inhibit inflammatory pathways, thereby providing a therapeutic target for treatment of chronic inflammatory diseases. In addition, research on the anticancer effects of *Entada rheedei* compounds has demonstrated, for some of the plant's isolated compounds, cytotoxicity against various cancer cell lines [11]. The action mechanisms include induction of apoptosis (progd cell death), inhibition of tumor growth, and inhibition of metastasis [14]. The potential finding of novel compounds with anticancer characteristics from *Entada rheedei* is extremely relevant for anticancer medicines in an age when cancer remains one of the top causes of death worldwide.

2. MATERIAL AND METHOD

2.1 Materials

The plant *Entada rheedei*, recognized for its indigenous medicinal significance, was gathered from the forest land of Pune, Maharashtra, India [15]. Due to its medicinal properties, it has been used in traditional medicinal practices for centuries. The source was obtained to initiate an investigation into possible bioactive compounds. The original plant material was accurately identified as *Entada rheedei*, during the authentication phase at the Botanical Survey of India[3]. Following authentication, the seed kernel component *Entada rheedei* was used for analysis. Before extraction and isolation, the seed kernels were milled and then prepared into a fine powder. The powder of the seed kernel served as the primary source materials to study the chemical constituents and potential biological activity. The study examines both

extraction and purification of compounds with characterization of the isolated compounds through validated methods and biological activity for screening possible medicinal application. The research provides evidence of the medicinal properties of *Entada rheedei* specifically for valuable bioactive compounds with possible potential in therapeutic development.

2.2 Soxhlet Extraction Method

To extract bioactive compounds from seeds of *Entada rheedei*, compounds were extracted using solvent extraction in a Soxhlet apparatus [16]. For extraction, 100 g of seeds were powdered, and the extraction was carried out successively using methanol, ethyl acetate and petroleum ether (pet ether) as solvents, which vary in their polarity. Methanol first extracted polar compounds, while ethyl acetate extracted less polar compounds and pet ether extracted non-polar compounds. The Soxhlet apparatus with a heating mantle boiled the solvent, condensing and recycling it through the plant material repeatedly up to 8 hours to fully extract the bioactive compounds in the range of polarity[17]. The method was repeated for each solvent, first with methanol, then ethyl acetate, and ultimately pet ether.

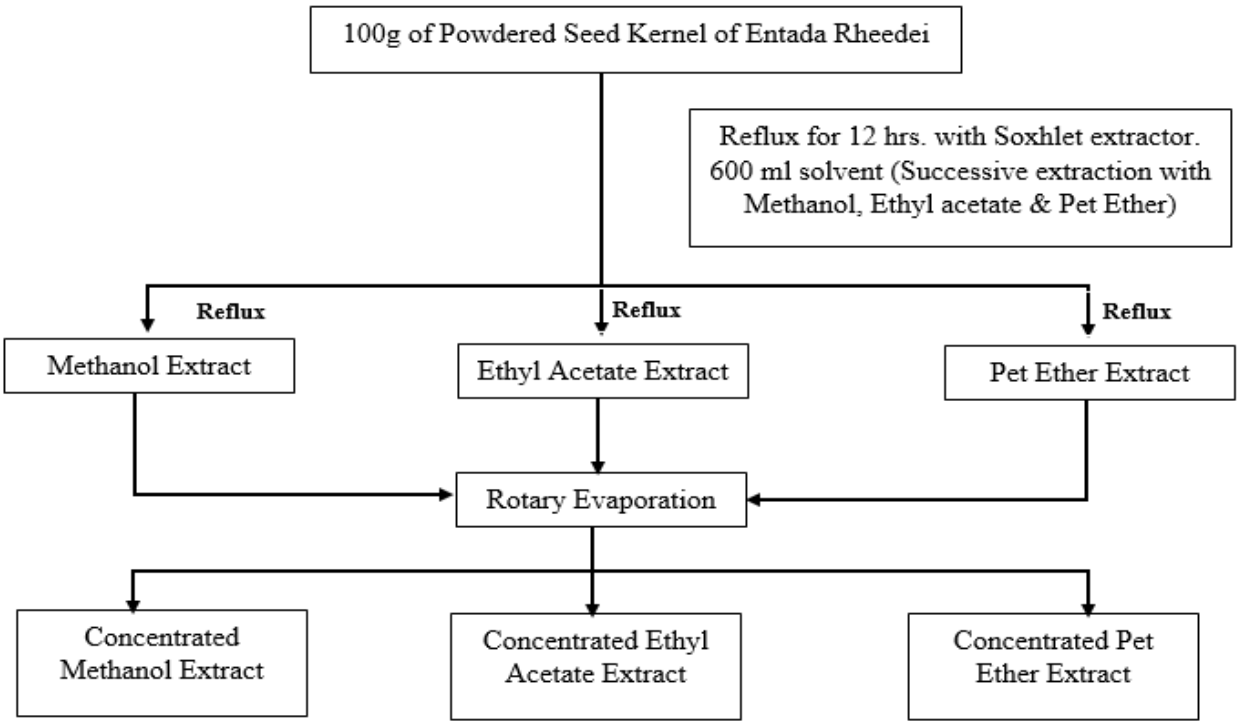


Fig.1 Soxhlet Extraction Process

Following the Soxhlet extraction method, the ethyl acetate extract weighed 7.64g, while the pet ether extract weighed 5.2 g. Biological activity and chemical composition assessments were carried out after the extracts were concentrated to remove the solvent [18]. Since the ethyl acetate extract is more polar, it is possible that a greater number of compounds were extracted due to the greater polarity of the ethyl acetate extraction. The pet ether extract, being less polar, indicates an abundance of some potentially hydrophobic compounds, such as alkaloids and flavonoids. In the column chromatography analysis that followed the isolation and characterisation of pure compounds from the ethyl acetate extract[19]. Column chromatography was utilised to separate physiologically active components from the ethyl acetate extract, yielding 0.40g of isolated substance. After separating components from the ethyl acetate extract, the pet ether extract underwent column chromatography, yielding an isolated compound weighing 2.8g. This equals a 5.2g increase in mass compared to the pet ether extract. The isolated compounds were confirmed as pure using Thin Layer Chromatography (TLC) with 7% ethyl acetate in pet ether as the mobile phase.

2.3 Column Chromatography

Column chromatography is a common technique used to separate and purify bioactive compounds from plant extracts [5]. The extraction and purification process we analyze in this study involves separating an isolated pure compound from *Entada rheedei*, using column chromatography. It commences with the column being prepared and requires packing a stationary phase of silica gel. A small volume of the plant extract will be dissolved in a solvent and added to the column

[19]. Subsequently, a gradient solvent will be utilized to elute the column, consisting of 100% petroleum ether through to 100% methanol, since separation of fractions requires a gradual increase in polarity. Fractions will then be collected and monitored by thin layer chromatography (TLC), pooled according to fractions having a similar retention factor (Rf) value, and further analyzed for an isolated and purified compound with subsequent characterization and biological activity assays performed to provide potential pharmacological uses.

3. CHARACTERIZATION OF ISOLATED PURE COMPOUND FORM *ENTADA RHEEDEI*

The fields of natural products chemistry and pharmacology have shown much interest in the extraction and separation of compounds from natural sources and their potential biological activities [20] . *Entada Rheedei* is a member of the Fabaceae family that has many biological activities, and therefore provides many extracts of purified compounds that may be of future therapeutic interests. This study examines biological activities, extraction and characterization of isolated a purified compound from *Entada Rheedei* [21]. High resolution mass spectrometry (HRMS), infrared (IR) spectroscopy and nuclear magnetic resonance (NMR) spectroscopies confirmed molecular properties of what was essentially purified to an orange oil [22]. This study aims to identify the chemical profile, functional groups, and biological activities of this purified compound [23].

^{1}H-NMR

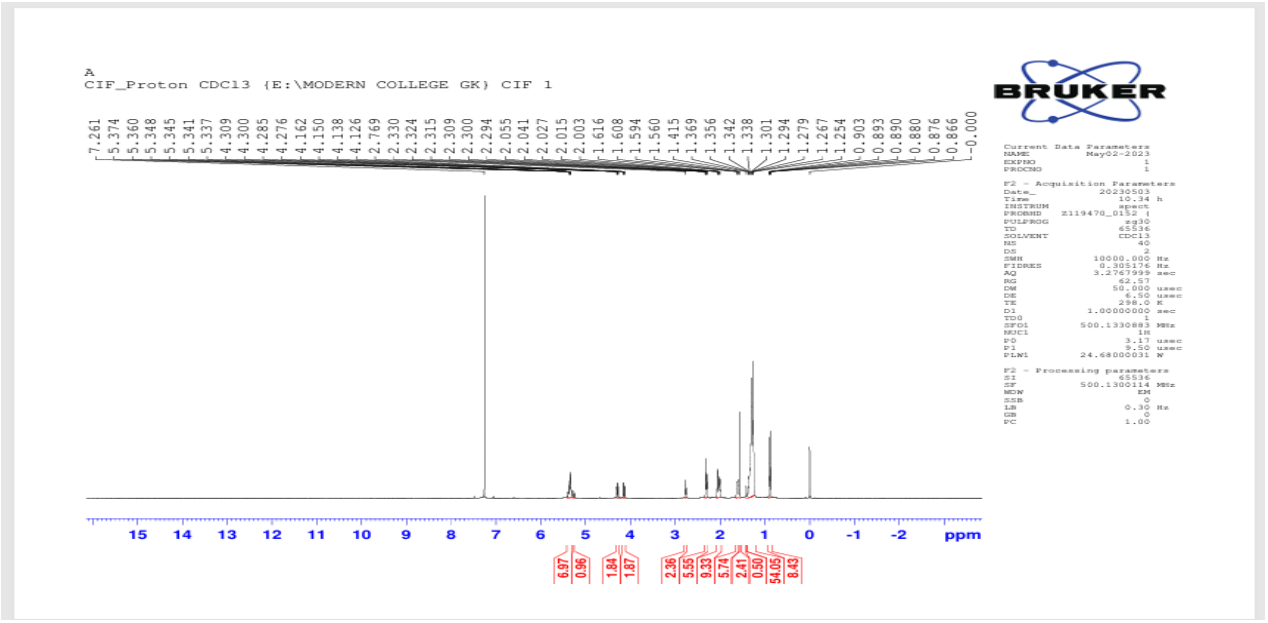


Fig.2 ^{1}H-NMR

In the 1H-NMR spectra (Fig. 2) of the isolated molecule in deuterated chloroform (CDCl3), a 7.26 ppm chemical shift was detected, generating peaks linked with the compound's functional groups and structures. A triplet at δ 0.89 ppm was assigned to the protons on a methyl group adjacent to a methylene carbon [24]. The chemical shifts of δ 1.25 and δ 1.60 abounded to aliphatic methylene (–CH2) protons. The δ 2.03 ppm shift corresponds to protons on a methylene group coupled to a C=C bond, whereas the signal at δ 2.32 ppm suggests protons on a methylene group bound to a carbonyl group. The δ 2.80 ppm signal represents methylene group protons with two C=C bonds. Furthermore, peaks at δ 4.28 and δ 4.12 ppm indicated the presence of a proton on an oxygen atom, whereas a peak at δ 5.34 ppm indicated olefinic protons. These changes indicated triglyceride and ester functional groups in the molecule.

^{13}C- NMR

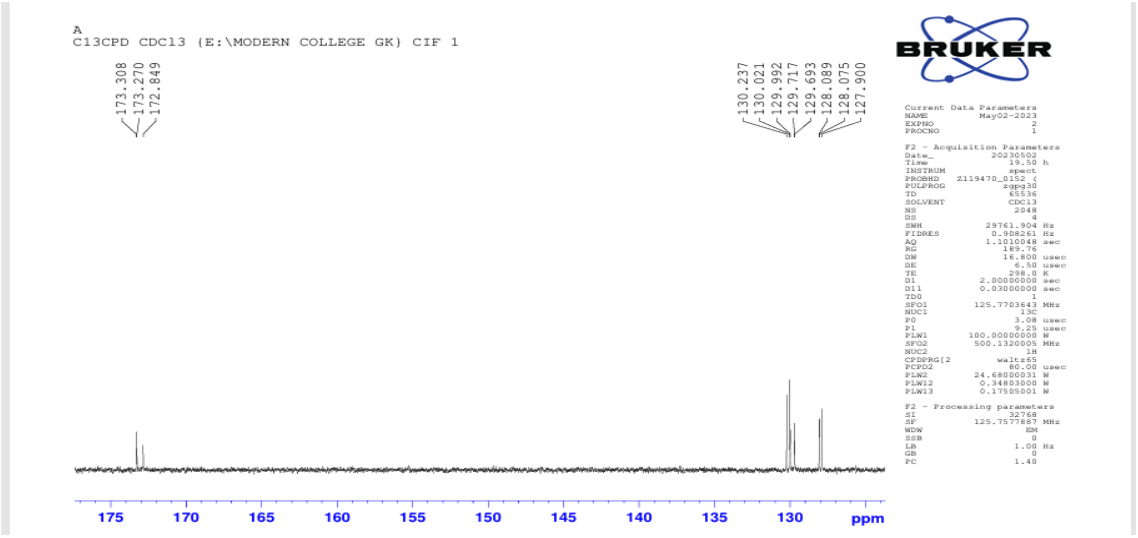


Fig.3 ^{13}C- NMR

The structure of the compound was further clarified by the ^{13}C-NMR spectrum (Fig. 3). C=C bonds were responsible for the chemical alterations observed in the δ 127.19–130.23 ppm range [25]. The ester functional groups' carbonyl quaternary carbons were found as strong signals at δ 173.1, 172.8, and 172.1 ppm. Signals ranging from δ 14.1-68.8 ppm show the presence of carbons such as methyl (-CH3), methylene (-CH2), and oxygenated methylene (-OCH2). These assignments are summarized in Table 1, and the carbon environment in the structure was consistent with that of a triglyceride-like compound.

COSY

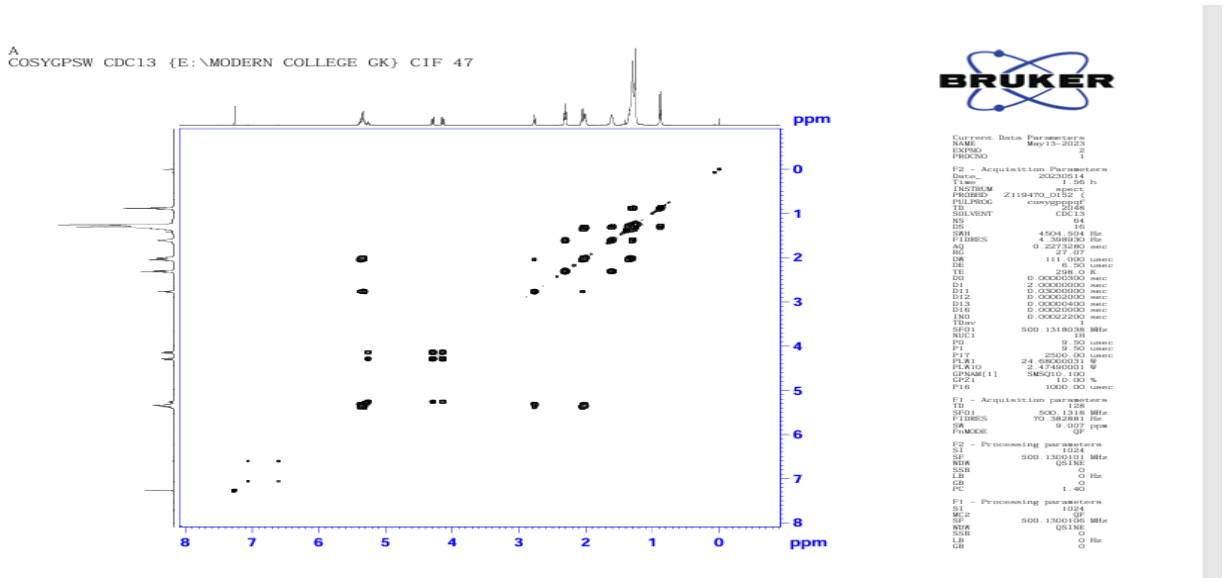


Fig.4 COSY

The proton-proton correlations in the compound were determined by the 2D COSY (Correlation Spectroscopy) spectrum (Fig. 4). The COSY spectrum demonstrated that the proton at δ 0.89 ppm (methyl group) was coupled to the protons at δ 1.25 ppm (methylene group), and in the same way, the protons at δ 1.25 ppm were coupled to those at δ 1.60 ppm. These correlations were instrumental in establishing the connectivity between the aliphatic chains and the various protons in the compound [26]. It was imperative to verify the proton connectivity of the isolated compound using the COSY spectrum.

DEPT

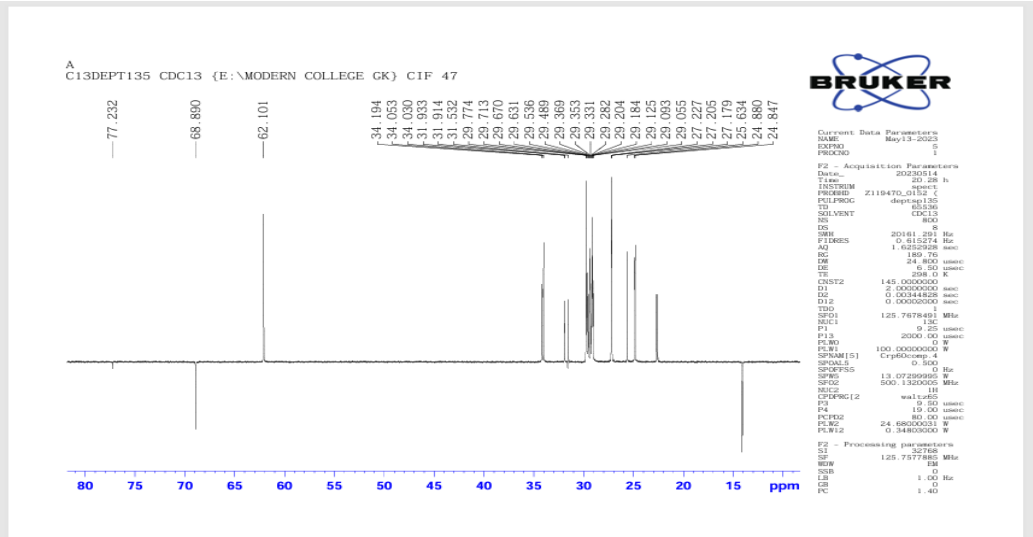


Fig.5 DEPT

The DEPT-135 spectrum (Fig. 5) was especially helpful in differentiating distinct classes of carbons of the compound you studied. The peaks at δ 14.1 ppm correspond to methyl (–CH3) carbons, while the methylene (–CH2) carbons are typically found around δ 24.8–62.1 ppm [27]. The olefinic methylene carbons of the compound are located at δ 127.9–130.9 ppm, whereas the oxygenated methine (–CH) was found at δ 68.8 ppm. This spectrum confirmed the ester group, the methylene chains, and the olefinic bonds, as suggested by the structure of the compound you studied [28].

HMBC

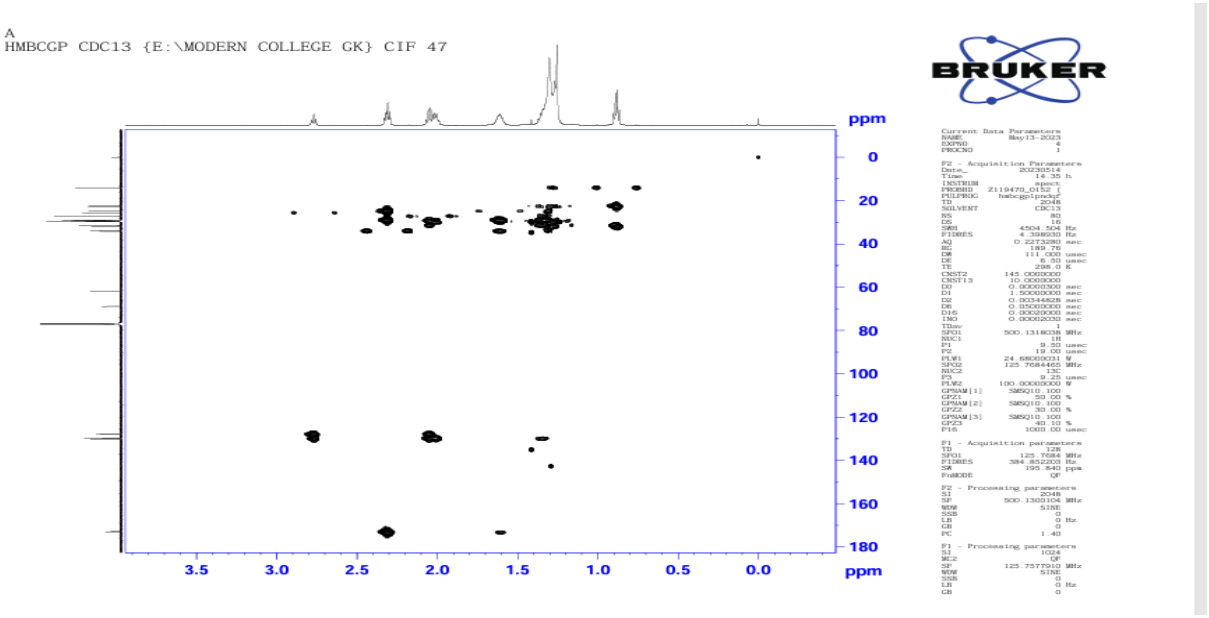


Fig.6 HMBC

The HMBC (Heteronuclear Multiple Bond Correlation) spectrum (Fig. 6) is crucial for the observation of long-range proton-carbon correlations[29]. The signal at δ 5.37 ppm coincided with carbons at δ 128 and 130 ppm, indicating conjugated double bonds in the molecule. The middle carbon, at δ 68.8 ppm, connects with methylene protons at δ 4.28

and 4.12 ppm and forms part of the glycerol backbone. Additionally, the carbonyl carbons (δ 173.1 and 172.1 ppm) from the ester functional groups were connected to the respective protons in the structure. These correlations throughout the HMBC spectrum clearly demonstrate the compound's connectivity.

HSQC

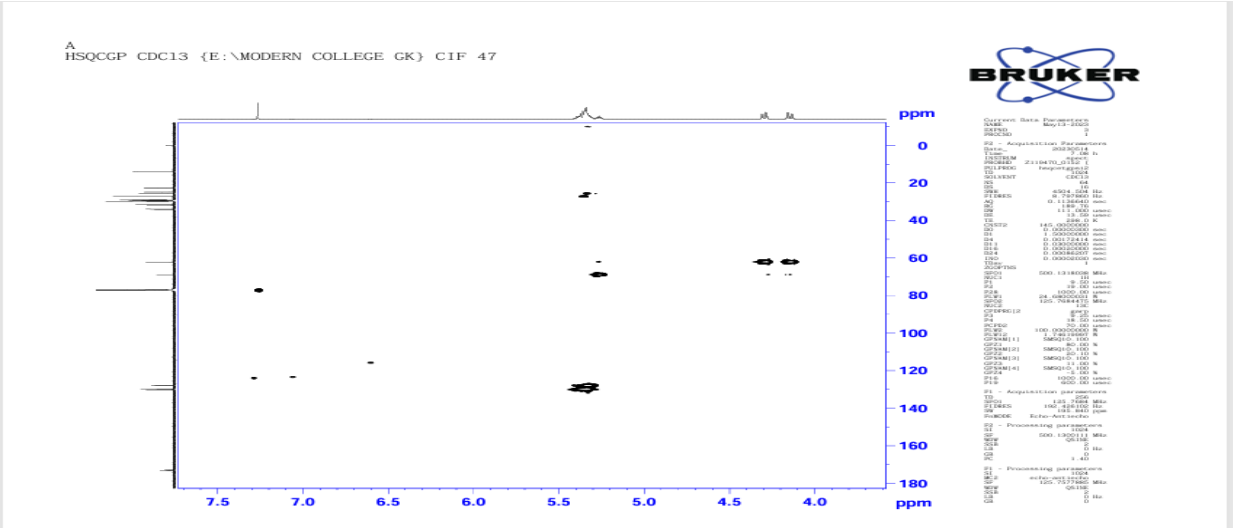


Fig.7 HSQC

The HSQC (Heteronuclear Single Quantum Coherence) spectrum (Fig. 7) provides important information regarding the correlations between protons and carbons [30]. The HSQC spectrum confirms the interactions of protons with their attached carbons. As an example, two peaks at δ 4.28 and 4.12 ppm in the proton spectrum showed correlations with the carbon at δ 68.8 ppm, representing the glycerol backbone of the triglyceride. Also, the proton signals correlated with the relevant corresponding carbon atoms in the structure, as the carbon peaks at δ 127.9–130.9 ppm are assigned as olefinic methane carbons.

FTIR

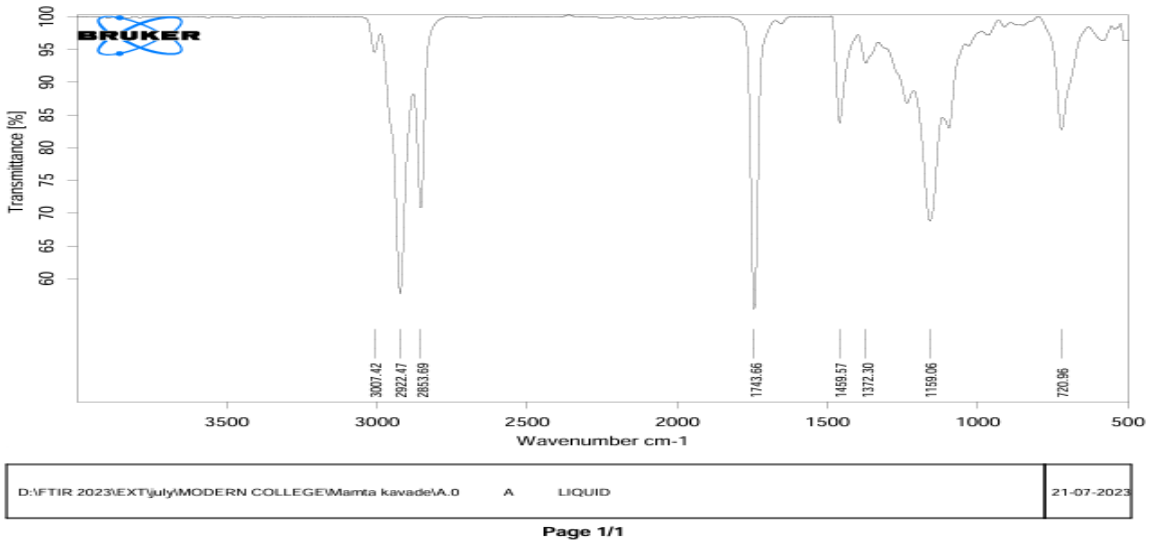


Fig.8 FTIR

The FTIR (Fourier Transform Infrared) spectra (Fig. 8) provided crucial information regarding the functional groups that are present in the compound [31]. The broad band at 1,745 cm-1 represents the carbonyl group's stretching resonance, which is typical of ester functional groups. In addition, the ester's C-O stretching vibration was responsible for the 1,163 cm-1 band. The C-H stretching vibrations of the methylene and methyl groups were matched by the bands at 2,922 and

2,852 cm-1, indicating the existence of long aliphatic chains within the structure. The molecule has conjugated double bonds, as seen by the peak at 3,000 cm-1, which corresponds to an alkene's C-H stretch.

LCMS

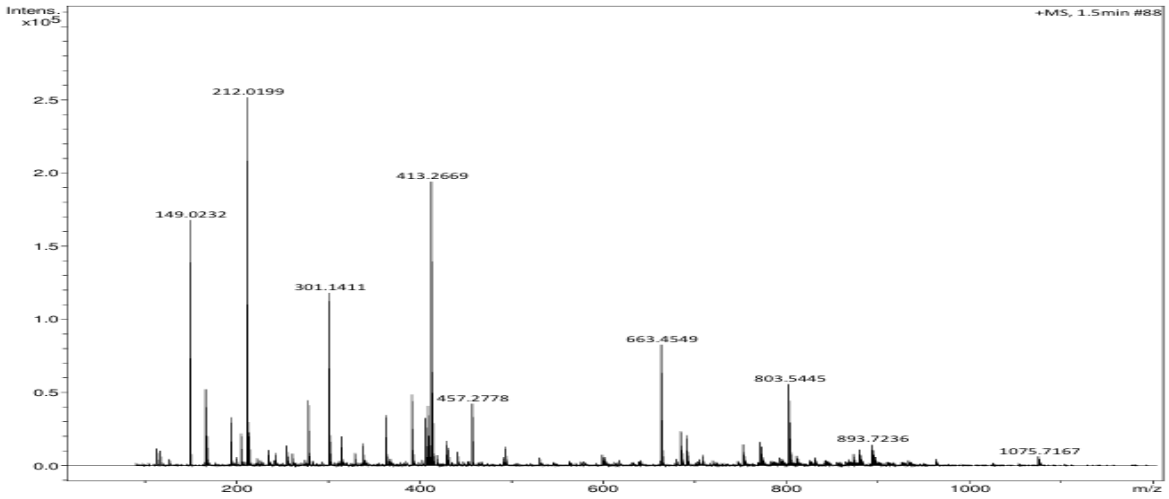


Fig.9 LCMS

The empirical formula of the chemical was C71H126O6 with a molecular weight of 1075.7830 g/mol, as determined by the LCMS data (Figure 9). The mass spectrometry findings confirmed the compound's purity, as did the NMR (nuclear magnetic resonance) and FTIR (Fourier-transform infrared) spectra.

The compound obtained from *Entada rheedei* has been well-defined chemically based on thorough chemical characterization of the compound using several different spectroscopic methods. The compound structure was described as a triglyceride-like ester with conjugated double bonds and long aliphatic chains. One of the strongest confirmations of the structure comes from the consistency of NMR, FTIR, and LCMS data which supported each other. Additional assessment is required to establish the biological activity of the reported compound, which might elucidate the medicinal components of *Entada rheedei.* The methods used in this research are necessary for the characterization of complex natural products to facilitate the discovery of new bioactive compounds.

Based on the above spectroscopic reports, the structure of the compound is

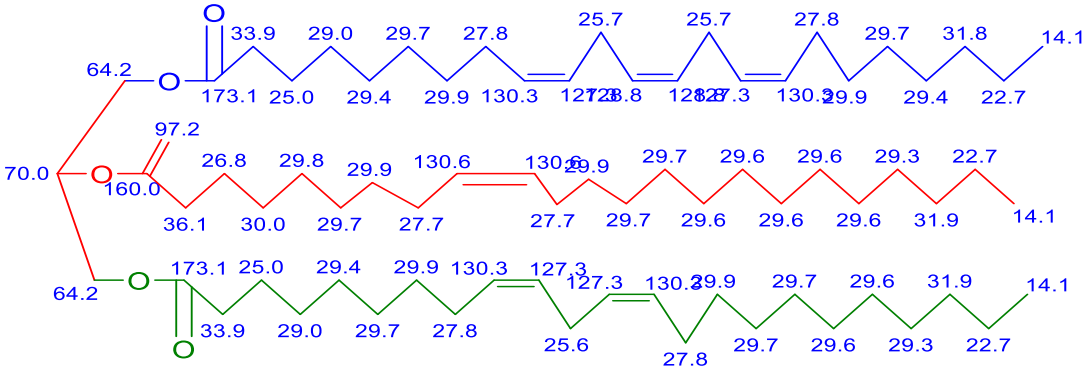


Fig.10 Structure Elucidation of Compound 1 (Triglyceride)

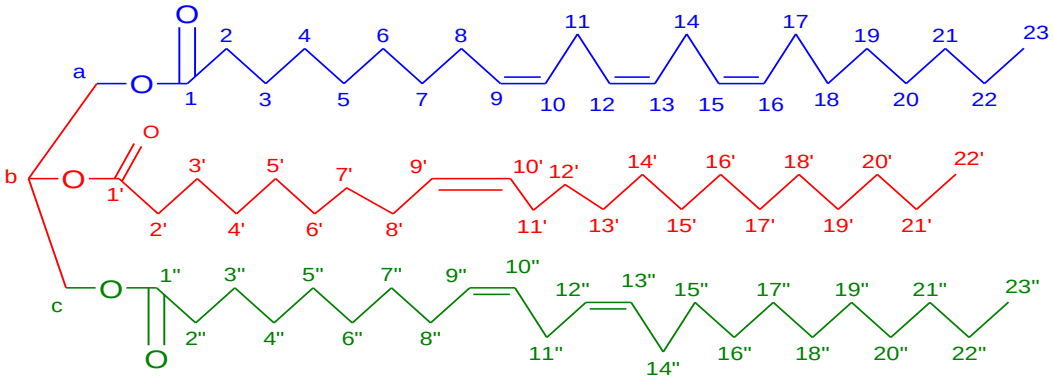
The image (Fig. 10) illustrates the structure elucidation of Compound 1, a triglyceride derived from *Entada rheedei*. The chemical shifts in the 13C-NMR data provide information about the structure by identifying the various forms of carbon present in the regions of chemical shifts. These exact shifts show that the triglyceride contains ester groups, longer aliphatic side chains, and conjugated double bonds. The blue, red, and green colors represent separate fatty acid side chains or vegetable parts of the triglyceride, illustrating individual structural changes. For example, the methylene and methine peaks from around 29.0–30.0 indicate long aliphatic chains, and δ 173.1 ppm chemical shift is indicative of carbonyls (C=O), which in triglycerides indicate esters. A major confirmation of conjugated double bonds is the double

bond carbon peaks region, δ 127.7–130.9 ppm, which correspond to olefinic carbons. In addition, the peaks in δ ranges of 97.2–160 ppm show that the backbone of the triglyceride is glycerol (C3H8O3). Collectively, the NMR information is also important in facilitating the identification of the structure of this compound, which is useful in examining the bioactive potential of *Entada rheedei.*

Table 1. Spectroscopic Data for the Analysis of *Entada Rheedei* Compound Using NMR Techniques

Position	H-NMR	13C-NMR	HMBC	HSQC
a	4.28, 4.12 (*d*)	62.1	5.37	4.28, 4.12
b	5.37(*dd*)	68.8	4.28, 4.12	5.37
c	4.28, 4.12 (*d*)	62.1	5.37, 2.32	4.28, 4.12
1, 1', 1"	-	172.8, 172.1, 173.1	5.34, 4.28, 4.12, 2.32,	-
2, 2', 2"	2.32 (m)	34.0, 34.1, 34.0	1.26	2.32
3,3',3"	1.26 2.32 (m)	24.8, 25.1, 24.8	2.32	-
4, 4',4"	1.33(m)	29.0	-	-
5,20,5',20',5",20"	1.34(m)	29.4	-	-
6,19,6',13',14',15',16' 17',18',19' 6",16",17"	1.35 (m)	29.6	-	-
7,18,7',12',7",15"	1.60 (m)	29.7. 29.8, 29.9	-	-
8,17, 8',11',8",14"	1.26(t)	27.2,27.1,27.2	-	-
9,16, 9',10',9"13",	5.30(d)	130.0	1.26	-
10,15,10",12"	5.30(d)	127	1.26	-
11, 14, 11"	1.26(t)	25.7	-	-
12, 13	5.30(d)	128	1.26	-
19, 13' 18",19', 16"	1.56 (m)	29.6, 29.5, 29.6	2.03	-
21,21',21"	2.03(m)	31.9	0.89	-
22,22',22"	1.25(m)	24.8	0.89,2.03	-
23,23'23"	0.89(m)	14.1	2.03	-

A compound extracted from *Entada Rheedei* was examined using several Nuclear Magnetic Resonance (NMR) techniques, including 1H-NMR, 13C-NMR, the chemical shifts and coupling constants for various positions (an, bn, cn, and so forth) were indicative of the compound's molecular structure. -H COSY, HMBC, and HSQC. The spectroscopic data is delineated in Table 1. The chemical shifts and coupling constants for various positions (an, bn, cn, and so forth) were indicative of the compound's molecular structure. For instance, position an exhibit a doublet in the H-NMR spectrum at 4.28 and 4.12 ppm, as well as a carbon shift of 62.1 ppm in the 13C-NMR. Also, the coupling patterns and correlations between protons and carbons are described for different positions to aid in characterizing the functional groups and connectivity of the compound. The careful analysis of the spectroscopic data assists in the structural characterization and understanding the biological activities of the compound.



Molecular Weight: 1073.8110

(9*Z*,12*Z*,15*Z*)-3-((9*Z*,12*Z*)-tricosa-9,12-dienoyloxy)-2-((*Z*)-tricosa-1,10-dien-2-yloxy)propyl tricosa-9,12,15-trienoate

Fig.11 Structure of compound 1.

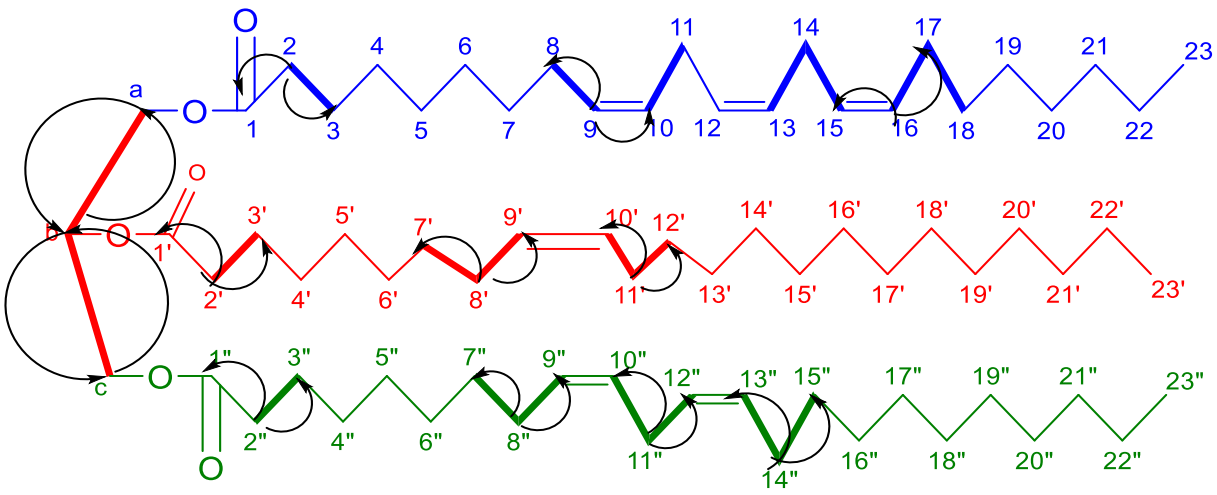


Fig.12 HMBC (arrow) and ^{1}H-^{1}H COSY (Bold)

Analysis of a compound extracted from *Entada Rheedei*, including its structure and detailed NMR analysis. The molecular structure is depicted in the first image (Fig. 11), with carbon positions labeled. The second image (Fig. 12) illustrates the interpretation of HMBC (arrows) and H-H COSY (bold lines) correlations. The molecular structure is composed of a complex chain of carbon atoms, with specific regions indicating functional groups. The 13C-NMR shifts observed in the compound's spectra are consistent with the carbonyl and carboxyl groups' location in the 172–178 ppm range. The glycerol backbone carbons exhibit a shift between 60–72 ppm, while unsaturated carbons are observed between 124–134 ppm. The characterization of this lipid-like compound is supported by the fact that most of the compound consists of aliphatic carbons, which are in the range of 10–35 ppm. The data compare very well with literature values and confirm the identity and structure of the compound[32].

4. RESULTS AND DISCUSSION

The antibacterial activity of the extracted purified chemical from *Entada rheedei* was evaluated against a diverse array of microorganisms, such as *Staphylococcus aureus*, *Bacillus subtilis*, *E. coli*, *Candida albicans*, *Aspergillus niger*, and *Proteus mirabilis*. Bacterial inhibition was minimally affected by the purified chemical when tested using the agar diffusion technique. Depending on the organism, the zone of inhibition ranged from 12 to 17 mm. The MIC values of 0.5 µl/mL for *E. coli*, *Aspergillus niger*, and *Staphylococcus aureus* were equivalent to those of commercial antibiotics such as fluconazole and streptomycin. Thus, the purified chemical exhibited antibacterial activity.

4.1 Antimicrobial Activity

Culture used for antimicrobial activity

In this study, the antimicrobial effects of the evaluated substances were examined by selecting a diverse array of microorganisms. Escherichia coli (NCIM2065), *Bacillus subtilis* (NCIM2063), *Staphylococcus aureus* (NCIM2178), and *Proteus mirabilis* (NCIM2388) were the bacterial isolates that were analysed. Each of these strains has links to different diseases. In addition, we decided to use the fungal strain *Candida albicans* (NCIM3100) due to its importance in human disease, particularly in patients that are immunocompromised. For broader diversity and to carry out efficacy tests against fungal pathogens, we included the filamentous fungus *Aspergillus niger* (ATCC504). Overall, the cultures we selected demonstrate the diversity of microorganisms selected, including fungi, gram-positive bacteria, and gram-negative bacteria.

Culture medium

The microorganisms were grown using many different types of culture media. The bacteria isolates were firstly subculture in nutrient broth which is nutrient-rich and supports the growth of a wide range of microorganisms. The bacteria were incubated at 37°C for 18 hours which is the optimum temperature for the growth of human-associated bacterial infections. In order to guarantee adequate growth, the fungal isolates were incubated in Sabouraud Dextrose Agar (SDA), a selective medium for fungi cultivation, at 25°C for 72 hours. The microorganisms were cultivated under optimal conditions by preparing the nutrient broth and SDA with meticulous attention to detail, as illustrated in Tables 2 and 3.

Table 2. Composition of Nutrient Broth

Ingredients	g/L
Peptones	10 g
Beef extract	1 g
Yeast extract	2 g
Sodium Chloride	5 g

The table 2. delineates the composition of Nutrient Broth, a prevalent medium employed for the cultivation of microorganisms and bacteria. It is made up of four main components: peptones (10 g/L), which are high in amino acids and nitrogen; beef extract (1 g/L), which adds growth factors and vitamins; yeast extract (2 g/L), which provides essential growth nutrients like B vitamins; and sodium chloride (5 g/L), which keeps the solution's osmotic balance.

Table 3. Composition of SDA

Ingredients	g/L
Mycological peptone (enzymatic digest of casein and animal tissues)	10 g
Dextrose	40 g
Agar	15 g

Sabouraud Dextrose Agar (SDA) is a culture medium that is frequently employed for the cultivation of fungi. The following table.3 details its composition. It contains 10 g of mycological peptone per liter, which is a source of nitrogen and essential nutrients. The principal carbon source for microbial proliferation is dextrose, which is present at a concentration of 40 g per Liter. The solution is solidified by the 15 g of agar in the medium, which enables the isolation and cultivation of fungal organisms. These components establish an environment that is conducive to the proliferation of fungi.

4.2 Antimicrobial Activity

The Agar diffusion method, which is widely recognised, was employed to conduct the antimicrobial activity test. This method is used to determine the minimum amount of an antibiotic necessary to impede the growth of microorganisms. A sterile cotton sample was submerged in a suspension of the test organism. In order to ensure that the microorganism was evenly distributed across the agar surface, the swab was subsequently used to inoculate the entire surface of the agar plate, first longitudinally and subsequently vertically. The agar surface was allowed to cure for five minutes after inoculation. Mueller-Hinton agar plates with a diameter of 4 mm were aseptically perforated using a cork borer that was autoclaved to ensure sterility. A wax stylus was used to mark the wells on the bottom of the Petri dish. The test solution was carefully injected into each well using a micropipette. The procedure was repeated for each well. Finally, the plates were incubated in an incubator set to 37°C for 24 to 48 hours.

Antimicrobial Activity of Pet Ether Extract

Table 4. Antimicrobial Activity of Ethyl Acetate and Pet Ether Extract

Sr. No.	Sample name	Zone of Inhibition (mm)					
		Microorganism studied					
		A	Mean	B	Mean	C	Mean
1.	Ethyl acetate extract	16, 15, 16, 17, 15	15.8	14, 15, 14, 14, 14	14.2	16, 15 16, 16, 15	15.6
2.	Pet Ether extract	13, 14, 13, 14, 13	13.4	13, 16, 14, 17, 15	15	13, 15, 14, 17, 15	14.8
3.	Antibiotic used		28		31		30

A: - Candida albicans (NCIM 3100), B: - E. coli (NCIM2065), C: - Aspergillus niger (ATCC504)

Table 4 shows the antibacterial activity of pet ether extract against three different sample categories (A, B, and C) and a variety of microorganisms. The concentration used in this experiment was 1 µl/ml. Bacteria were treated with 10 µg/ml streptomycin, and fungi with 10 µg/ml fluconazole. The average inhibition zone for the ethyl acetate extract was 15.8 mm in sample A, 14.2 mm in sample B, and 15.6 mm in sample C. Pet ether was used to examine the inhibition zone averages and zones. The averages for samples C, B, and A were 14.8 mm, 15 mm, and 13.4 mm, respectively. The inhibition zones

of the three samples were much bigger for the antibiotic employed as a control, with averages of 28 mm, 31 mm, and 30 mm, respectively. This means that the pet ether extract has modest antibacterial activity as compared to the antibiotic.

Table 5. Antimicrobial Activity of Ethyl Acetate and Pet Ether Extract

Sr. No.	Sample name	Zone of Inhibition (mm)					
		Microorganism studied					
		D	Mean	E	Mean	F	Mean
1.	Ethyl acetate extract	13, 14, 14, 15, 15	14.2	13, 12, 11, 12, 12	12	15, 15, 15, 15 15	15
2.	Pet Ether extract	17, 16, 15, 15, 16	15.8	11, 12, 13, 12, 13	12.2	16, 13, 16, 16, 16	15.4
3.	Antibiotic used		21		26		25

D: - *Staphylococcus aureus* (NCIM2178), E*: - Bacillus subtilis* (NCIM2063), F*: - P. mirabilis* (NCIM2388)

The average zone for each of the microorganisms, as well as the zone of inhibition measured in mm against a range of microbes for each of the samples tested, are presented in the table. The samples tested were an antibiotic standard, a pet ether extract, and an ethyl acetate extract. The mean inhibition zone size for Microorganisms D, E, and F, using ethyl acetate extract, was 14.2mm, 12mm, and 15mm, respectively. Comparatively, the mean inhibition zone size with pet ether extract for D, E, and F, respectively was 15.8mm, 12.2mm and 15.4mm, indicating pet ether extract had slightly greater activity than ethyl acetate extract. The antibiotic control sample had significantly larger inhibition zones for all of the organisms, with mean inhibition zone sizes for D, E, and F being 21 mm, 26 mm, and 25 mm.

Antimicrobial Activity of Isolated Pure Compound

Table 6. Antimicrobial Activity of Isolated Pure Compound

Sr. No.	Sample name	Zone of Inhibition (mm)					
		Microorganism studied					
		A	Mean	B	Mean	C	Mean
1.	Isolated Compound	13, 14, 15, 15, 15	14.4	14, 15, 15, 14, 14	14.4	14, 15, 15, 15 15	14.8
	Antibiotic used		28		31		30
		D		E		F	
1.	Isolated Compound	17, 16, 16, 15, 16	16	13, 12, 13, 12, 13	12.6	16, 15, 16, 16, 15	15.6
	Antibiotic used		21		28		25

A: - *Candida albicans* (NCIM 3100), B: - *E. coli* (NCIM2065), C: - *Aspergillus niger* (ATCC504), D: - *Staphylococcus aureus* (NCIM2178), E*: - Bacillus subtilis* (NCIM2063), F*: - P. mirabilis* (NCIM2388)

Zone of inhibition (mm) can be used to determine the antimicrobial activity of a purified compound isolated against some microorganisms. The compound was utilized at 1µl/ml, and streptomycin (10µg/ml) against bacteria and fluconazole (10µg/ml) against fungi. Table shows results for six microorganisms:5 *Candida albicans* (A), *E. coli* (B), *Aspergillus niger* (C), *Staphylococcus aureus* (D), *Bacillus subtilis* (E), and *Proteus mirabilis* (F). For *Candida albicans* (A) against the isolated compound, the average zone of inhibition was 14.4 mm. For *E. coli* (B), the isolated compound averaged 14.4 mm. A mean zone of inhibition of 14.8 mm was calculated for the fungal organism, *Aspergillus niger* (C). The isolated compound inhibited *Staphylococcus aureus* (D) at 16 mm, *Bacillus subtilis* (E) at 12.6 mm, and *Proteus mirabilis* (F) at 15.6 mm. The antibiotics (fluconazole and streptomycin) had much larger inhibition zones ranging from 21 mm-31 mm across the tested bacteria and fungi.

IC Values of Extracts and Pure Compound

Table 7. MIC Values of Extracts and Pure Compound

Sr. No.	Compound	MIC values in µl/mL					
		Microbial strain					
		Candida albicans	***E. coli***	***Aspergillus niger***	***Staphylococcus aureus***	***Bacillus subtilis***	***Proteus mirabilis***
1	Ethyl acetate extract	-	0.5	0.5	0.5	-	0.5
2	Pet ether extract	-	1	1	1	-	0.5
3	Pure compound	-	0.5	0.5	0.5	-	0.5

(-) Indicates MIC Value is too Low to be determined

The table displays the Minimum Inhibitory Concentration (MIC) values for a purified chemical and several extracts in relation to various microbial strains. The MIC value for ethyl acetate extract against *E. coli*, *Aspergillus niger*, *Staphylococcus aureus*, and *Proteus mirabilis* is 0.5 µl/mL. Pet ether extract has MIC values of 1 µl/mL for *E. coli*, *Aspergillus niger*, *Staphylococcus aureus*, and 0.5 µl/mL for *Proteus mirabilis*. The purified substance has equivalent MIC values to the ethyl acetate extract, with 0.5 µl/mL for *E. coli*, *Aspergillus niger*, *Staphylococcus aureus*, and *Proteus mirabilis*. MIC values for *Candida albicans* and *Bacillus subtilis* were not determined, denoted by "-".

The table displays the Minimum Inhibitory Concentration (MIC) values of a purified compound and a variety of extracts against a variety of microbial strains. The ethyl acetate extract has MIC values of 0.5 µl/mL for *E. coli*, *Aspergillus niger*, *Staphylococcus aureus*, and *Proteus mirabilis*. In contrast, the MIC values of pet ether extract are 1 µl/mL for *E. coli*, *Aspergillus niger*, and *Staphylococcus aureus*, and 0.5 µl/mL for *Proteus mirabilis*. The MIC values of the purified compound are comparable to those of the ethyl acetate extract, with a value of 0.5 µl/mL for *E. coli*, *Aspergillus niger*, *Staphylococcus aureus*, and *Proteus mirabilis*. The MIC values for *Candida albicans* and *Bacillus subtilis* were not determined, as indicated by the symbol "-".

4.3 Antioxidant activity of Isolated compound by DPPH Assay

DPPH Assay

a) Determination of DPPH free radical scavenging activity by IC_{50} method.

An in vitro antioxidant experiment was carried out to determine the isolated compound's ability to scavenge free radicals. The IC_{50} was determined using modified versions of the standard DPPH test techniques. The extracts were produced at various concentrations for HE, EAE, and ME, including BHT (20, 40, 60, or 100 µg/mL) and HE, EAE, and ME (20, 60, 100, or 500 µg/mL). Each test or standard sample was prepared using the procedure outlined below: The volume was increased to 4 mL by adding methanol to 1 mL of 0.1 mM DPPH in methanol. This mixture was prepared by combining 1 mL of DPPH solution in methanol with 1 mL of plant extract or standard. The reaction mixture was left at room temperature for 30 minutes in the absence of light. The absorbance at 515 nm was measured using a UV-Vis spectrophotometer. To ensure accuracy, each extract's IC_{50} was tested three times. The IC_{50} was used to compute the percentage of DPPH free radical inhibition, which is represented by the following formula.

$$\% \text{ inhibition} = \frac{Ac - As}{Ac} \text{x } 100$$

Where, AC represents the absorbance of the control (0.802).

A_s = absorbance of sample/compound

Table 8. Antioxidant Activity of Ethyl Acetate Extract by DPPH Assay

Concentration	Absorbance			Average	%RSA
	1	2	3		
20 µg/ml	0.762	0.762	0.762	0.762	5
40 µg/ml	0.706	0.706	0.706	0.706	12
60 µg/ml	0.642	0.642	0.642	0.642	20
100 µg/ml	0.521	0.521	0.521	0.521	35
500 µg/ml	0.080	0.080	0.080	0.080	90

The antioxidant activity of the ethyl acetate extract was measured using the DPPH assay, as shown in Table 8. The data indicate that the percentage of radical scavenging activity (%RSA) increases dose-dependently. The RSA percentage was 5% at the lowest concentration (20 µg/ml). This increased to 12% at 40 µg/ml and 20% at 60 µg/ml. At 100 µg/ml, antioxidant activity dramatically enhanced, leading to a 35% boost in RSA. The maximum concentration (500 µg/ml) resulted in the greatest %RSA at 90%, showing the extract's antioxidant capability. In addition, the absorbance values were consistent among replicates confirming the validity of the results. These findings indicate the extract's potency in scavenging free radicals in a concentration dependent manner.

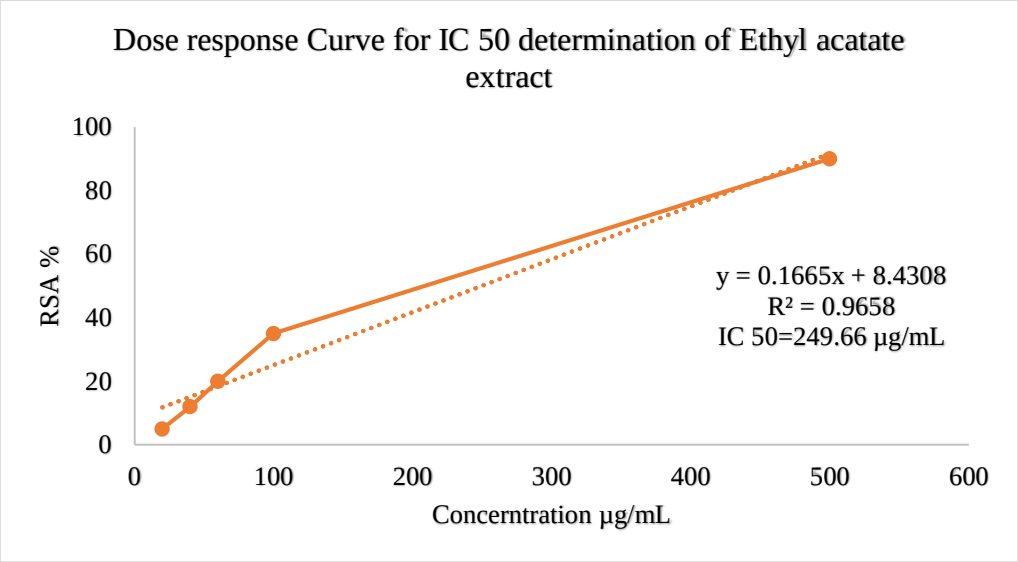


Fig.13 Dose Response Curve for IC 50 Determination of Ethyl Acetate Extract

Figure 13 shows a linear rise in radical scavenging activity (%RSA) with concentration in the dose-response curve for calculating the IC_{50} of the ethyl acetate extract. The regression equation, y = 0.1665x + 8.4308, and the high correlation coefficient (R^2 = 0.9658) indicate a strong association. The IC_{50} value of 249.66 µg/mL indicates the concentration required for 50% RSA. The extract exhibited maximum scavenging activity (90% at 500 µg/mL), confirming its potent antioxidant properties in a concentration-dependent manner.

Table 9. Antioxidant activity of Pet ether extract by DPPH assay

Concentration	Absorbance			Average	%RSA
	1	2	3		
20 µg/ml	0.765	0.765	0.765	0.765	4.61
40 µg/ml	0.720	0.721	0.720	0.720	10.22
60 µg/ml	0.680	0.680	0.680	0.680	15.21
100 µg/ml	0.630	0.630	0.631	0.630	21.45
500 µg/ml	0.350	0.350	0.315	0.350	56.36

In order to ascertain the antioxidant activity of the petroleum ether extract, Table 9 displays the results of the DPPH assay. The extract's ability to scavenge free radicals was quantified as percentage radical scavenging activity (%RSA) in the

DPPH experiment. The %RSA of the petroleum ether extract increased from 4.61% to 56.36% when the concentration was increased from 20 µg/ml to 500 µg/ml in the DPPH experiment. This indicates that antioxidants have a concentration-dependent potential, with higher concentrations exhibiting an enhanced free radical scavenging capacity. Absorbance readings obeyed a similar decreasing trend that Laboratory results indicated higher concentration treatments provided greater DPPH reduction potential activity. In the DPPH, the extract rate of 500 µg/ml demonstrated the highest antioxidant activity, suggesting its potential for antioxidant activity. These findings suggest that the petroleum ether extract had the capacity to mitigate oxidative stress by scavenging radicals.

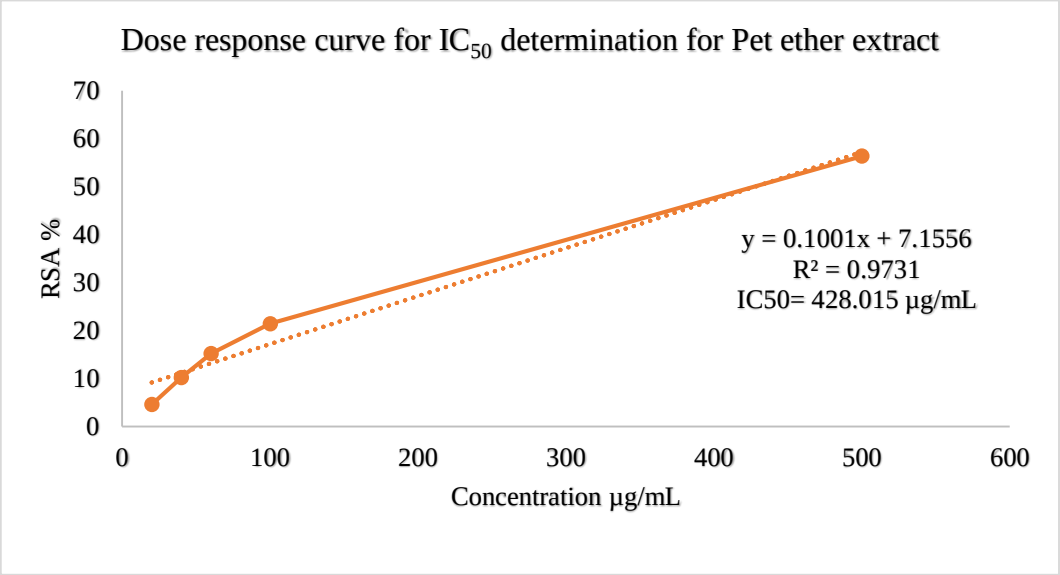


Fig.14 Dose Response Curve for IC_{50} Determination for Pet Ether Extract

Fig. 14 displays the dose-response curve for the IC_{50} determination of the petroleum ether extract, which demonstrates the correlation between concentration and radical scavenging activity (%RSA). The linear regression equation, y = 0.1001x + 7.1556, with a R^2 value of 0.9731, suggests a robust correlation between concentration and %RSA. The concentration necessary to inhibit 50% of DPPH radicals is referred to as the IC_{50} value, which is 428.015 µg/mL. The increasing trend in %RSA with higher concentrations highlights the extract's antioxidant potential. This data suggests that the extract exhibits moderate free radical scavenging activity, with potency increasing at higher concentrations.

Table 10. Antioxidant activity of isolated compound

Concentration	Absorbance			Average	%RSA
	1	2	3		
20 µg/ml	0.706	0.706	0.706	0.706	12
40 µg/ml	0.577	0.577	0.577	0.577	28
60 µg/ml	0.465	0.465	0.465	0.465	42
100 µg/ml	0.321	0.321	0.321	0.321	60
500 µg/ml	0.040	0.040	0.040	0.040	95

The antioxidant activity of the isolated chemical was tested at various doses, with absorbance measurements taken at each level. The findings, as shown in Table 10, indicate that the percentage of radical scavenging activity (%RSA) rises concentration-dependently. At 20 µg/ml, the %RSA was 12%, gradually increasing to 28% at 40 µg/ml and 42% at 60 µg/ml. A notable increase was observed at 100 µg/ml, where %RSA was 60%. Likewise, the highest concentration tested, 500 µg/ml, had an impressive 95% RSA, which also demonstrated strong antioxidant potential. The reproducible absorbance values of the triplicates provide evidence of a reliable data set. These results indicate the compound is effective at neutralizing free radicals, which has great potential for antioxidant applications.

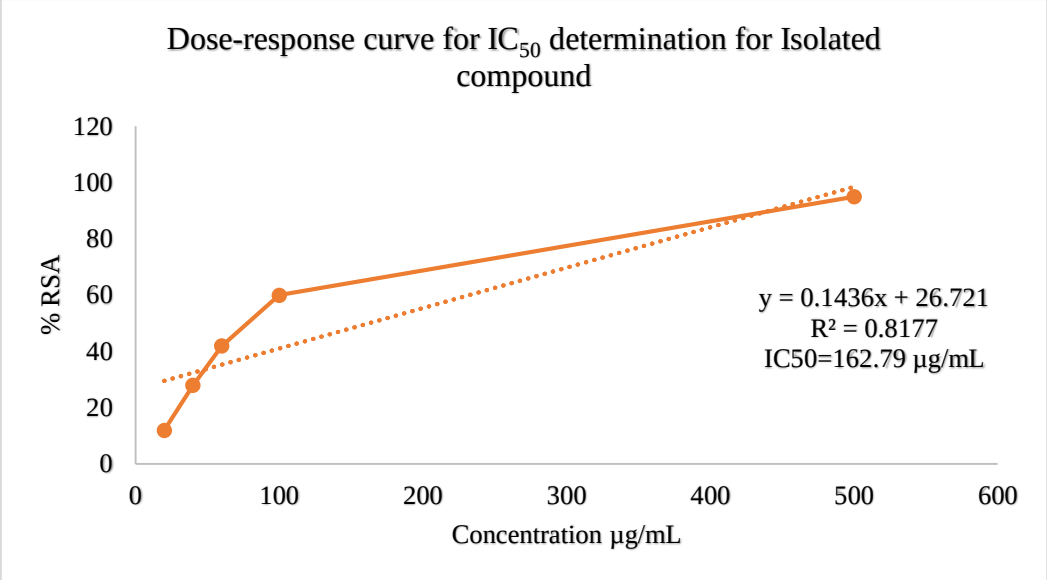


Fig.15 Dose Response Curve for IC_{50} Determination for Pet Ether Extract

Displayed in the dose-response curve below is the antioxidant activity of the isolated compound, with %RSA increasing linearly according to concentration. The linear regression equation y = 0.1436x + 26.721 shows a positive association between %RSA and concentration, with an R2 value of 0.8177 indicating a good match. The IC_{50} value of 162.79 µg/mL indicates 50% radical scavenging activity. As shown in the dose-response curve, the %RSA generally increased with increased concentration and reached about 95% RSA at 500 µg/mL. These data suggest that the compound demonstrates powerful antioxidant capacity and continues to increase antioxidant activity up to higher concentrations. The IC_{50} for the Pet Ether Extract is promising for future pharmaceutical and nutraceutical studies.

4.4 IC_{50} Value

Sr. No.	Sample	IC_{50} Value (µg/mL)
1	Ethyl acetate extract	249.66
2	Pet ether extract	428.01
3	Isolated pure compound	162.79

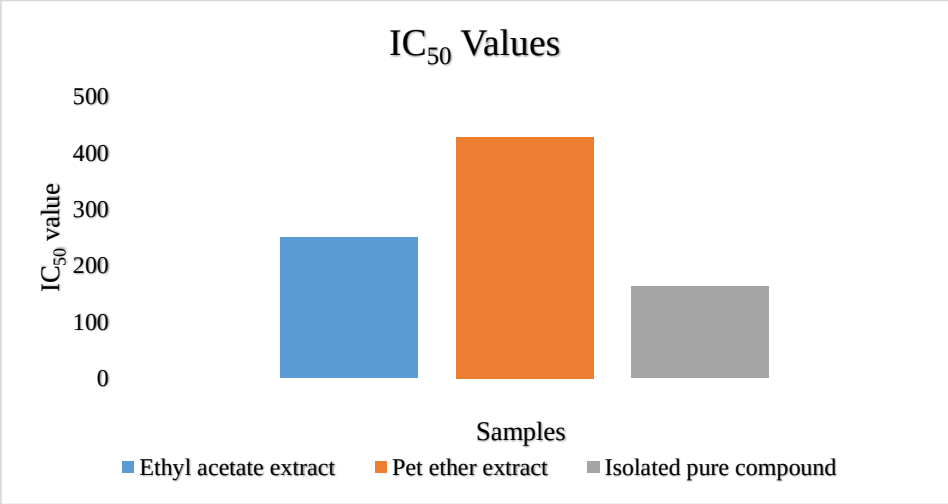


Fig. 16 IC_{50} Value

The IC_{50} values of the extracts suggest their potential for inhibition, with lower values implying better efficacy. The pure isolate exhibited the highest inhibition potency at 162.79 µg/mL, while the ethyl acetate extract followed at 249.66 µg/mL and the pet ether extract at 428.01 µg/mL exhibited the lowest inhibition potential. The bioactivity of the isolated purified compound was substantially greater than that of plant extracts, which suggests that it will be a promising candidate for the isolation of additional pharmacological effects.

The IC_{50} range of treatments provided evidence for effectiveness while suggesting that it may indicate the isolated and purified bioactive compounds were relatively more effective than plant extracts demonstrating varying potencies. This suggests that extraction procedures play a critical role in isolating bioactive compounds.

4.5 Anticancer Activity

The sterile experimental study utilized 96-well plates, sterile 5 mL pipettes, and a multichannel pipette to transfer correct amounts of reagents. A CO_2 incubator was employed to keep cell culture conditions stable. Dulbecco's Modified Eagle Medium (DMEM) served as the custodial substance. This medium required L-glutamine, non-essential amino acids (NEAA), fetal bovine serum (FBS), and antibiotics to maintain a sterile environment, such as streptomycin and penicillin. The MTT reagent was used in the cytotoxicity experiment.

Cell Culture and Growing Conditions

The National Centre for Cell Sciences in Pune, India, contributed the MCF-7 cell lines. The cell lines were grown in DMEM with 10% (v/v) fetal bovine serum (FBS), 1% L-glutamine, 1% non-essential amino acids (NEAA), 1% penicillin, and 1% streptomycin. Cell lines were cultured in a humid incubator at 37°C with 5% CO_2 to maximise growth and viability.

Preparation of Test Compound and Dilutions

The study compound, which was DMSO (tissue-culture grade) diluted and the starting compound concentration was 250 µM. Serial dilution was done to derive the concentrations used to test to determine if there was a possible cytotoxic factor on the MCF-7 cell line.

Assay Protocol

Eight distinct concentrations of the synthesized compound were administered to MCF-7 cells, with the maximum concentration being 250 micromolar. Cells were grown and plated at a density of 1×10^3 per well on a 96-well plate. Each component's concentration was measured in triplicate. Following treatment, the cells were cultured for 48 hours under normal conditions. MTT formazan crystals produced after treating cells with a 5 mg/mL MTT solution for 4 hours. Following the elimination of the medium, the crystals were dissolved in DMSO, a solvent that is suitable for tissue culture. The OS chemist utilised a Read well Touch Automatic ELISA Plate Reader (Robotnik India Private Limited) to measure absorbance at 570 nm after 30 minutes of incubation. The vitality of cells treated with cell-free extracts and the standard medication Adriamycin (ADR) was expressed as a percentage of untreated control cells (which were presumed to be 100% alive).

CONCLUSION

This work involved the extraction, isolation and characterization of a pure compound from *Entada Rheedei* for evaluation of biological activities, namely antioxidant and antimicrobials. Moderate antioxidant activity was observed with the ethyl acetate extract and mild activity was found with the pet ether extract. In contrast, the isolated pure compound exhibited substantial antioxidant activity showing an IC_{50} value of 162.79 µg/mL compared to the ethyl acetate extract (249.66 µg/mL) and pet ether extract (428.01 µg/mL) reflecting a higher capacity for free radical scavenging. Biological activity was assessed on MCF-7 (breast cancer) cell lines and demonstrated no significant cytotoxic activity, as cells continued to grow at each concentration tested. Because of the high IC_{50} values with MCF-7 cells, we encourage further studies involving the pure compound and other cancer cell lines to confirm a broader anticancer activity. A greater literature review is needed to provide more evidence for the previously reported anticancer activity of *Entada Rheedei* extracts and their potential pharmacological importance.

The antimicrobial properties of the obtained pure compound were assessed against selected bacterial and fungal strains. The isolated compound showed moderate inhibitory activity, with inhibition zones ranging from 12.6 mm to 16 mm. However, this activity was lower in comparison to a standard antibiotic (streptomycin) and a standard antifungal agent (fluconazole). The isolated compound inhibited *E. coli*, *Aspergillus niger*, *Staphylococcus aureus*, and *Proteus mirabilis* at 0.5 µl/mL, according to minimum inhibitory concentration (MIC) study. Several spectroscopic analyses (NMR, FTIR, LCMS, COSY, DEPT, HMBC, and HSQC) confirmed the isolated compound's structure, which was a triglyceride-like ester with conjugated double bonds and long aliphatic chains. Overall, the research implies that the extracted pure molecule needs additional pharmacological investigation, particularly into its potential as an antioxidant or antibacterial

agent. Future work to support this conclusion would include examining structural modifications to increase potency, bioavailability evaluations, and toxicity assessments to determine its applicability for therapeutic purposes.

REFERENCES

[1] S. S. Tatma, B. P. Soni, N. R. Fefar, S. B. Jhala, K. V. Gadhvi, and M. B. Shah, "Entada Adans, an ethnopharmacologically important genus: A review".

[2] R. R. Deokar, S. R. Mane, S. R. Kamble, and S. R. Patil, "A NEW LOCALITY RECORD FOR AN ENDANGERED PLANT SPECIES ENTADA RHEEDEI SPRONG. (MIMOSACEAE) IN SANGLI DISTRICT, MAHARASHTRA," *World J. Pharm. Res.*, vol. 6, no. 17.

[3] M. Okba, "Entada rheedii seeds thioamides, phenolics, and saponins and its antiulcerogenic and antimicrobial activities," *J. Appl. Pharm. Sci.*, pp. 101–108, May 2018, doi: 10.7324/JAPS.2018.8513.

[4] M. A. Sufian, M. S. A. Mehedi, M. A. Rashid, and M. R. Haque, "BIOLOGICAL INVESTIGATION OF ENTADA RHEEDII SPRENG. AND ISOLATION OF ENTADAMIDE A FROM ITS SEED," *Int. Res. J. Pharm.*, vol. 6, no. 7, pp. 411–414, Jul. 2015, doi: 10.7897/2230-8407.06785.

[5] Q.-W. Zhang, L.-G. Lin, and W.-C. Ye, "Techniques for extraction and isolation of natural products: a comprehensive review," *Chin. Med.*, vol. 13, no. 1, p. 20, Dec. 2018, doi: 10.1186/s13020-018-0177-x.

[6] K. Patel, D. Patel, D. Panchal, K. Patel, and D. U. Upadhyay, "A Review on High Performance liquid Chromatography," vol. 10, no. 10, 2022.

[7] A. a Sridhara, "Antiproliferative Effect of Entada rheedii Crude Lectin Extract on Human Colorectal Cancer Cells," *Jordan J. Biol. Sci.*, vol. 17, no. 03, pp. 541–548, Sep. 2024, doi: 10.54319/jjbs/170317.

[8] K. S. Salim, A. Harun, N. A. Aziz, S. Daud, and S. Z. M. So'ad, "BIOACTIVE PHYTOCHEMICALS AND PHARMACOLOGICAL FACETS OF," vol. 28, no. 5, 2024.

[9] S. Tilvi, M. S. Majik, and K. S. Singh, "Mass Spectrometry for Determination of Bioactive Compounds," in *Comprehensive Analytical Chemistry*, vol. 65, Elsevier, 2014, pp. 193–218. doi: 10.1016/B978-0-444-63359-0.00008-2.

[10] A. Aydemir, "Skill-based immigrant selection and labor market outcomes by visa category," in *International Handbook on the Economics of Migration*, A. F. Constant and K. F. Zimmermann, Eds., Edward Elgar Publishing, 2013. doi: 10.4337/9781782546078.00033.

[11] L. K. Nzowa *et al.*, "Rheediinosides A and B, two antiproliferative and antioxidant triterpene saponins from Entada rheedii," *Phytochemistry*, vol. 71, no. 2–3, pp. 254–261, Feb. 2010, doi: 10.1016/j.phytochem.2009.10.004.

[12] F. Rachmadita, E. Martati, S. N. A. Syed Mohamad, and S. Z. Mat So'ad, "Antimicrobial Study of Chloroform Fraction from the Leaves of Entada spiralis Ridl.," *J. Pharm.*, vol. 1, no. 1, pp. 45–53, Jan. 2021, doi: 10.31436/jop.v1i1.33.

[13] S. Naik and S. Kumar, "Biochemical Characterization of Lactose Binding Entadin Lectin from *Entada rheedii* Seeds with Cytotoxic Activity against Cancer Cell Lines," *ACS Omega*, vol. 5, no. 27, pp. 16430–16439, Jul. 2020, doi: 10.1021/acsomega.0c00577.

[14] J. Dumortier, M. Carriquiry, and A. Elobeid, "Impact of climate change on global agricultural markets under different shared socioeconomic pathways," *Agric. Econ.*, vol. 52, no. 6, pp. 963–984, Nov. 2021, doi: 10.1111/agec.12660.

[15] S. S. Rahangdale and S. R. Rahangdale, "Floristic diversity of Bhimashankar Wildlife Sanctuary, northern Western Ghats, Maharashtra, India," *J. Threat. Taxa*, vol. 9, no. 8, p. 10493, Aug. 2017, doi: 10.11609/jott.3074.9.8.10493-10527.

[16] L. R. Nair, M. Balasubrahmanian, and Department of Biotechnology, Vivekanandha College of Arts and Sciences for Women (Autonomous) Tiruchengode, Namakkal, Tamil Nadu, India., "Correlation Between Phytochemicals and Antioxidant Activities of Different Leaf Extracts of Entada rheedii," *J. Plant Sci. Res.*, vol. 39, no. 1, pp. 199–208, Jun. 2023, doi: 10.32381/JPSR.2023.39.01.20.

[17] F. Rachmadita, "ANTI MICROBIAL STUDY OF CHLOROFORMFRACTIONOFENTADA SPIRALIS RIDL. LEAVES," 2020.

[18] M. A. López-Bascón and M. D. Luque De Castro, "Soxhlet Extraction," in *Liquid-Phase Extraction*, Elsevier, 2020, pp. 327–354. doi: 10.1016/B978-0-12-816911-7.00011-6.

[19] M. D. Luque De Castro and F. Priego-Capote, "Soxhlet extraction: Past and present panacea," *J. Chromatogr. A*, vol. 1217, no. 16, pp. 2383–2389, Apr. 2010, doi: 10.1016/j.chroma.2009.11.027.

[20] A. M. Clark, "[No title found]," *Pharm. Res.*, vol. 13, no. 8, pp. 1133–1141, 1996, doi: 10.1023/A:1016091631721.

[21] F. Mariani, "Biological activities of phenolic compounds extracted from some plant species from east Kalimantan province, Indonesia," Doctoral Degree, Chulalongkorn University, Bangkok, Thailand, 2018. doi: 10.58837/CHULA.THE.2018.19.

[22] M. V. Palmeira-Mello, A. R. Costa, L. P. De Oliveira, O. Blacque, G. Gasser, and A. A. Batista, "Exploring the potential of ruthenium(II)–phosphine–mercapto complexes as new anticancer agents," *Dalton Trans.*, vol. 53, no. 26, pp. 10947–10960, 2024, doi: 10.1039/D4DT01191K.

[23] G. K. Jayaprakasha, L. Jagan Mohan Rao, and K. K. Sakariah, "Chemistry and biological activities of C. longa," *Trends Food Sci. Technol.*, vol. 16, no. 12, pp. 533–548, Dec. 2005, doi: 10.1016/j.tifs.2005.08.006.

[24] K. A. N. P. Bandara, V. Kumar, U. Jacobsson, and L.-P. Molleyres, "Insecticidal piperidine alkaloid from Microcos paniculata stem bark," *Phytochemistry*, vol. 54, no. 1, pp. 29–32, May 2000, doi: 10.1016/S0031-9422(00)00025-X.

[25] H. J. Jaffer, Y. A. Aldhaif, and I. H. R. Tomi, "Synthesis, characterization of new carboxylic acid derivatives bearing 1,3,4-thiadiazole moiety and study their liquid crystalline behaviors," *Mol. Cryst. Liq. Cryst.*, vol. 643, no. 1, pp. 199–215, Jan. 2017, doi: 10.1080/15421406.2016.1266881.

[26] J. H. Schuur, P. Selzer, and J. Gasteiger, "The Coding of the Three-Dimensional Structure of Molecules by Molecular Transforms and Its Application to Structure-Spectra Correlations and Studies of Biological Activity," *J. Chem. Inf. Comput. Sci.*, vol. 36, no. 2, pp. 334–344, Jan. 1996, doi: 10.1021/ci950164c.

[27] V. H. R. De Souza, S. A. Silva, L. P. Ramos, and S. F. Zawadzki, "Synthesis and Characterization of Polyols Derived from Corn Oil by Epoxidation and Ozonolysis," *J. Am. Oil Chem. Soc.*, vol. 89, no. 9, pp. 1723–1731, Sep. 2012, doi: 10.1007/s11746-012-2063-5.

[28] I. Ahmad, W. Tan, Q. Ali, and H. Sun, "Latest Performance Improvement Strategies and Techniques Used in 5G Antenna Designing Technology, a Comprehensive Study," *Micromachines*, vol. 13, no. 5, p. 717, Apr. 2022, doi: 10.3390/mi13050717.

[29] K. Backholer *et al.*, "Australia in 2030: what is our path to health for all?," *Med. J. Aust.*, vol. 214, no. S8, May 2021, doi: 10.5694/mja2.51020.

[30] Y. Jayasubba Reddy, V. Agarwal, A. Lesage, L. Emsley, and K. V. Ramanathan, "Heteronuclear proton double quantum-carbon single quantum scalar correlation in solids," *J. Magn. Reson.*, vol. 245, pp. 31–37, Aug. 2014, doi: 10.1016/j.jmr.2014.05.004.

[31] S. A. Khan, S. B. Khan, L. U. Khan, A. Farooq, K. Akhtar, and A. M. Asiri, "Fourier Transform Infrared Spectroscopy: Fundamentals and Application in Functional Groups and Nanomaterials Characterization," in *Handbook of Materials Characterization*, S. K. Sharma, Ed., Cham: Springer International Publishing, 2018, pp. 317–344. doi: 10.1007/978-3-319-92955-2_9.

[32] E. Alexandri, R. Ahmed, H. Siddiqui, M. Choudhary, C. Tsiafoulis, and I. Gerothanassis, "High Resolution NMR Spectroscopy as a Structural and Analytical Tool for Unsaturated Lipids in Solution," *Molecules*, vol. 22, no. 10, p. 1663, Oct. 2017, doi: 10.3390/molecules22101663.