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The Biological activities of Aqueous and Alcoholic of leaf extract of *Ziziphus mauritiana*

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ABSTRACT

The study's findings revealed that the extraction process involved using methanol, ethanol, and water with both green and dried leaves of Z. *mauritiana*. Additionally, three different concentrations of methanol and ethanol (100%, 70%, and 50%) were utilized, resulting in noteworthy bioactive qualities. The phytochemical investigation identified several substances, including glycosides, tannins, phenols, flavonoids, resins, coumarins, and saponins, which may contribute to its therapeutic qualities. The methanol green cold extract at 100% and the ethanol green hot extract at 70% showed the most potent antibacterial activity against *Staphylococcus epidermis*. Both the MIC and MBC values demonstrate potent inhibitory effects against specific microorganisms. The findings indicate that Z. *mauritiana* has the potential to serve as a valuable source of bioactive chemicals that may offer health advantages. Additional investigation is necessary to thoroughly examine its therapeutic capabilities and potential uses in medicine and healthcare. The presence of phenolic substances and flavonoids could be ascribed to the characteristics. Z. *mauritiana* has bioactive components that have been found to have antimicrobial and antioxidant activities. This makes it a promising candidate for further investigation in developing natural antibacterial and antioxidant agents. Moreover, the results of this study indicate that Z. *mauritiana* has the potential to serve as a natural substitute for synthetic antibiotics and antioxidants. However, additional research is required to investigate its medicinal capabilities thoroughly.

Keyword: *Ziziphus mauritiana*, MIC , antibiotics , antioxidants.

النشاط الحيوي للمستخلص المائي والكحولي لأوراق نبات *Ziziphus mauritiana*

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مستخلص:

كشفت نتائج الدراسة أن عملية الاستخلاص تضمنت استخدام الميثانول والإيثانول والماء مع كل من الأوراق الخضراء والمجففة لنبات *Ziziphus mauritiana*. بالإضافة إلى ذلك، تم استخدام ثلاثة تركيزات مختلفة من الميثانول والإيثانول (100٪، 70٪، و 50٪)، مما أدى إلى صفات نشطة بيولوجيا جديرة بالملاحظة. حدد التحقيق الكيميائي النباتي عدة مواد، بما في ذلك الجليكوسيدات والعفص والفينول والفلافونويد والراتنجات والكومارين والصابونين، والتي قد تساهم في صفاته العلاجية. أظهر مستخلص الميثانول الأخضر البارد بنسبة 100٪ ومستخلص الإيثانول الأخضر الساخن بنسبة 70٪ أقوى نشاط مضاد للجراثيم ضد المكورات العنقودية البشرة. تُظهر كل من قيم *MIC* و*MBC* تأثيرات مثبطة قوية ضد كائنات دقيقة محددة. تشير النتائج إلى أن نبات *Z. mauritiana* لديه القدرة على العمل كمصدر قيم للمواد الكيميائية النشطة بيولوجيًا والتي قد توفر فوائد صحية. من الضروري إجراء تحقيق إضافي لإجراء فحص شامل لقدراته العلاجية واستخداماته المحتملة في الطب والرعاية الصحية. يمكن أن يعزى وجود المواد الفينولية والفلافونويدات إلى الخصائص. يحتوي *Z. mauritiana* على مكونات نشطة بيولوجيًا وجد أن لها أنشطة مضادة للميكروبات ومضادات الأكسدة. وهذا يجعله مرشحًا واعدًا لمزيد من البحث في تطوير عوامل طبيعية مضادة للبكتيريا ومضادات الأكسدة. علاوة على ذلك، تشير نتائج هذه الدراسة إلى أن نبات *Z. mauritiana* لديه القدرة على العمل كبديل طبيعي للمضادات الحيوية الاصطناعية ومضادات الأكسدة. ومع ذلك، هناك حاجة إلى مزيد من البحث للتحقق من قدراته الطبية بدقة.

الكلمات المفتاحية: السدر التفاحي ، التركيز المثبط الأدنى ، مضادات حيوية ، مضادات أكسدة.

INTRODUCTION

Plants have been the primary source of medicinal remedies for humans since ancient times and continue to be so in the present period. Pharmaceuticals, primarily synthetic medications, currently hold the primary position in modern medicine. However, phytomedicinal drugs, which are mainly derived from plants, enjoy more popularity. According to the World Health Organisation (WHO), in 1996, around 80% of the global population relied on plant-derived medications, especially in developing nations. Nevertheless, the process of developing drugs from plants is highly intricate and costly. Each new drug requires an investment of approximately 100-360 million US dollars and a minimum of 10 years of rigorous work involving various disciplines such as botany, chemistry, pharmacology, biomedical sciences, biotechnology, and even anthropology (Rates, 2001). Earth is home to around 250,000 to 500,000 distinct plant species. However, people and animals only consume 10% of these species. This indicates that conducting screenings of medicinal plants for bioactivity is beneficial to acquiring the essential knowledge required to develop innovative drugs. Plants generate two fundamental components: primary compounds, such as carbohydrates, proteins, and chlorophyll, and secondary compounds, commonly called phytochemicals, such as phenolic compounds, terpenoids, and flavonoids. The bioactive properties of certain plants are ascribed to their secondary or phytochemical compounds. These bioactive phytochemical compounds hold great potential for treating several human ailments, including diabetes, heart disease, infections, and cancer. Immunomodulation is a complex process that occurs when chemicals, bacteria, trauma, foreign objects, surgery, or ionizing radiation cause damage to any tissue or organ. This process entails the liberation of several bioactive compounds, including histamine, serotonin, and prostaglandins. (Morrow and Jackson-Robert, 2011) Opioids, non-steroidal anti-inflammatory drugs (NSAIDs), and traditional analgesics all derive from natural substances that have been utilized for many centuries. Salicin, a bitter glycoside included in willow bark extract, has been acknowledged since the 18th century for its capacity to alleviate pain and lower

fever. As far back as the 19th century, acetylsalicylic acid (ASA), an artificial derivative, was employed in medicine. *Ziziphus mauritiana*, often called Indian jujube or Ber, belongs to the Rhamnaceae family. Ziziphus plants have a global distribution. Z. *mauritiana*, a tropical shrub, is native to the Indian Subcontinent, Southeast Asia, Iran, and specific African regions. It is utilized in medicine and nutrition in many forms.

Throughout history, leaves have been utilized for their astringent properties and as an anti-typhoid agent (Akhtar et al., 2016; Najafi, 2013). Several studies have established that Z. *mauritiana* possesses several health benefits, including anti-cancer, hepatoprotective, anti-microbial, anti-diarrheal, and anti-diabetic qualities (Lim, 2013). The present work assessed the antibacterial properties of the methanol leaf extract of Z. *mauritiana*, a plant species found in Iraq.

METHODOLOGY

Botanical specimens : In July 2023, the leaves of Z. *mauritiana* (Fig. 1) were collected at the Al Gherai'at garden nurseries in Baghdad. After being verified and authenticated by Plant Taxonomist Dr. Hadil Radawi Hussein, the voucher specimen was stored at the Herbarium of the Laboratory Sciences Department of the College of Sciences at Al Mustansiria University. Once the leaves were gathered, they were thoroughly washed using tap water, followed by a second rinse with diluted water. Subsequently, they were left to dry in a shaded area for a maximum duration of seven days.

The dried leaves were pulverized into a fine powder using a GEEPAS® blender model GCG 292 and kept for future use. In addition, incorporate green foliage as well. Figure 1 displays the leaves of the Z. *Mauritian* plant.

Figure(1) *Ziziphus Mauritian* leaves

Extraction: The extraction process encompassed various techniques, including:

1- Microwave-assisted extraction (MAE): A solution was prepared by

combining 20g of powdered dry leaves with 200ml of sterilized distilled water in an Erlenmeyer flask (Tranetal, 2020). Subsequently, the flask was hermetically sealed using a layer of parafilm and subjected to microwave irradiation for 10 minutes. The solution was filtered using Whatman filter paper No. 1 from Whatman International Ltd., U.K. The filtrate was subsequently transferred to a BINDER GmbH incubator in Germany, which was maintained at a temperature of 45 °C. The filtrate was then left to evaporate for a maximum duration of 10 days. A robust extract was generated and employed in the research. A hot green leaf extract was prepared by combining 35 grams of finely chopped green leaves with 200 milliliters of sterilized distilled water in an Erlenmeyer flask. Subsequently, the flask was hermetically sealed using a layer of parafilm and subjected to microwave irradiation for a duration of 10 minutes (Kumar, 2021).

No text was provided. The filtrate was introduced into the incubator and treated consistently with the previous dry method. Alcohol (specifically methanol and ethanol) was extracted from dry and green leaves using the following procedure: 20 grams of dried leaves were ground into a powder, and 35 grams of chopped leaves were placed into separate Erlenmeyer flasks. A total of 200 milliliters of alcohol, consisting of methanol and ethanol, were poured into the flask at three distinct concentrations: 100%, 70%, and 50%. The flask was then sealed with a layer of parafilm and microwaved for three minutes. The solution was filtered using Whatman filter paper No. 1, manufactured by Whatman International Ltd. in the U.K. The filtrate was subsequently transferred to a BINDER GmbH incubator in Germany, which was maintained at a temperature of 45 °C. The filtrate was then left to evaporate for a maximum period of 10 days. A somewhat solid extract was generated and employed in the research. The microwave's power output was 2,450 MHz (Megawati et al., 2019).

2- The maceration technique : Maceration is a simple and cost-effective method of extraction that just requires a basic container. However, this approach is time-consuming. The extraction of dry and green leaves was successfully achieved using maceration procedures, employing methanol, ethanol at three different ratios (100%, 70%, and 50%), and distilled water.

The maceration process was conducted in a light-free environment using a rotary shaker for three days. The solutions were extracted and transferred to the incubator, similar to the previous microwave method.

Conducting a qualitative examination of specific bioactive components found in plant extracts.

Glycosides: The detection is accomplished by combining 2ml of Benedict's reagent with 1ml of the plant extract in a test tube. The solution is vigorously shaken and then placed in a boiling water bath for 5 minutes. Subsequently, the tube is allowed to cool, and observing a red precipitate confirms the presence of glycosides.

Tannins: The experiment involved adding a few drops of a 1% FeCl3 solution to a test tube holding 0.5 ml of the extract. The observation of a bluish-green color indicated the presence of tannins (Burns, 1971). The detection was accomplished by employing a ferric chloride solution, which was generated by dissolving ferric chloride salt in distilled water at a concentration of 1%. When this reagent is introduced to the extract in the watch glass that contains phenolic compounds, it produces a color that is either green or blue (Harbone, 1973).

Phenols: The detection was accomplished by utilizing a ferric chloride solution, which was generated by dissolving ferric chloride salt in distilled water at a concentration of 1%. When this reagent is introduced to the extract in the watch glass that contains phenolic compounds, it produces a color that is either green or blue (Harbone, 1973).

Flavonoids: The detection solution is prepared by combining 10 ml of 50% ethyl alcohol with 10 ml of a solution containing 50% potassium hydroxide (KOH). Equal amounts of this solution and the plant extract are then mixed. The presence of flavonoids is indicated by the appearance of a yellow color (Jaffer et al., 1983).

Saponin: The test is conducted by adhering to the two techniques outlined below:

A solution composed of plant extract dissolved in water. The solution was transferred into a test tube and vigorously agitated. The presence of soap can be confirmed by a thick and long-lasting foam, followed by adding 1% hydrochloric acid (HCl) when the foam remains stable for an extended period (Harborne, 1984).

A 1-3 ml volume of a 1% solution of

mercuric chloride (HgCl2) is added to 5 ml of the plant extract. The appearance of a white precipitate serves as proof of a positive detection.

Coumarins: The presence of coumarin was identified based on the information provided (Geisman, 1962). A small amount of plant extract was poured into test tubes for each previously stated extract. The tubes were coated with filter papers dampened with a diluted sodium hydroxide (NaOH) solution. Subsequently, the tubes were immersed in a water bath heated to boiling briefly. Following this, the filter papers were subjected to an ultraviolet light source. A vivid hue of greenish-yellow signifies the existence of coumarin.

Polymers: We employed the technique outlined in Shihata's publication from 1951 to identify resins. Specifically, we utilized 10 ml of each extract and added 20 ml of distilled water that had been acidified for detection. The detection process involved drying the mixture using heat.

Microorganisems: The antimicrobial activity testing utilized four pathogenic bacterial strains, including *Staphylococcus aureus, Staphylococcus epidermis, Klebsiella, Escherichia coli*, and one type of fungi, *Candida albicans*. The pathogenic strains were acquired from the Pathology Laboratory of Medical City and Laboratory Al-Kindy General Hospital in Baghdad. Professor Dr. Ali Haider Abdul_Hadi Alsakini identified them. The origins of these infections and their drug susceptibility profiles are given in Table 2. The mentioned type culture strain was obtained from the Department of Laboratory of Advanced Bacteriological Studies Sciences at the College of Sciences, Mustansiriyah University, in the Republic of Iraq.

Antimicrobial Assay: The antibacterial efficacy of Z. *mauritiana* leaves was assessed using different extracts, including methanol, ethanol, and water extracts obtained from both green and dry leaves. The extracts were prepared using both hot and cold methods. The method of dissemination was pursued by Well (Hussein &Hasan, 2022).

In the bacterial sensitivity test for plant extract, a volume of 0.1 ml of bacterial culture was applied to the Müller Hunton steel medium using a cork borer to create holes with a diameter of 6 mm. Then, five holes were made for each type of microbiology. Next, 0.1 ml of plant extract and various concentrations were added to each hole as speci-

fied in the previous paragraph. Furthermore, a sample of distilled water was introduced into one of the pits to serve as a negative control. Allow the dishes to remain undisturbed for 15 minutes, followed by incubation at 37.5 degrees Celsius for 24 hours. The inhibition zone is quantified using a ruler.

Fourier-Transform Infrared Spectroscopy (FTIR): By measuring the extract absorption of infrared radiation over a range of wavelengths (e.g., 50 to 5000 cm-1 wavenumber), Fourier transform infrared spectroscopy (FTIR) is used to identify the functional groups (e.g., amide, phosphate, carbonate, and hydroxyl) present in organic and inorganic compounds." The interferogram is gathered and digitized, and the Fourier transform is applied before the FTIR spectrum is shown on a contemporary FTIR spectrometer. An optical microscope and an FTIR spectrophotometer are coupled to create a device to perform FTIR microspectroscopy. The successful application of FTIR microscopes and I.R. micro spectrophotometers to various research fields (pharmaceuticals, polymers, semiconductors, and forensics) has made this technique a potent instrument for analyzing small samples. Numerous aspects of site creation processes, including chronology, the utilization of space, ancient pyrotechnologies, diagenesis, and the alteration of the archaeological record, are addressed using FTIR analysis in archaeological study(Berna, 2017).

GC-MS: A GC-MS analysis was performed to find the bioactive components in the ziziphus extracts. Using an H.P. (5977E) USA detector, the Agilent 7820A gas chromatograph was used to examine the sample. Using helium as the carrier gas and an all-glass injector operating in split mode, the sample was delivered at a linear speed of 32 cm/s. The HP-5 fused silica capillary column (30 m length x 250 µm inner diameter x 0.25 µm film thickness) was employed. The temperature program was as follows: 55 ˚C to 180 ˚C, 180˚C to 280˚C, 280˚C to 300˚C, 300˚C hold to 5 min.. Utilizing computer searches in commercial libraries, the components were identified.

Statistical Analysis : Values were expressed as means ± S.E. Comparisons between means were carried out using one-way ANOVA followed by LSD (least significant difference) and Tukey multiple comparison tests. The

$P < 0.05$ was accepted as significant in all statistical tests. SPSS software (version 17) was used to carry out all these tasks.

RESULTS

The results of the preliminary chemical detection showed that the Z. *mauritiana* plant contains many active ingredients, the most important of which are alkaloids and include Spinanina, jujube responsible for the anti-microbiology effectiveness, Our results agree with (shkhaier, et al, 2020), and also contains flavonoids of all kinds, including antioxidant glycosides, phenols, saponins, pectin, fats, tannic acid, glyphic acid, tannins and terpenes (table 1), and this is consistent with As for the nature of the extracts, they were characterized by a viscous texture, dark green color and aromatic smell, and the appearance of green color is attributed to the pigment chlorophyll and xanthine. The distinctive aromatic smell of Z. *mauritiana* can be attributed to the Sidr, which contains volatile oils. It also contains mucous substances, vegetable gels, and glue. The results showed that all types of bacteria were sensitive to the aqueous and alcoholic extract of the plant (Hameed, et al, 2020). The table (2,3,4,5,6) found that the cold aqueous extract of dry and green Z. *mauritiana* leaves gave the highest inhibition of Staphylococcus epidermis bacteria, 14.33mm.

In contrast, the cold aqueous extract, significantly, gave the highest inhibition of *Staphylococcus aureus*, 15.33. mm. It was found that cold methanol extract of green Z. *mauritiana* leaves at a concentration of 100% significantly gave the highest inhibition on *Staphylococcus epidermis* 24mm, while. The methanol extract at a concentration of 70% green leaves gave the highest inhibition against the bacteria *E. coli* 17.33mm, and the results of 70% ethanol alcohol green extract showed significantly higher inhibition against S. *epidermis* bacteria19.67mm.

	detection of effective groups							
detection type	dry hot D.W(soxhlet)	D.W green hot (microwave)	dry cool water	ethanol 100% hot dry (soxhlet)	ethanol 100% cool dry	ethanol 100% green hot (microwave)	methanol 100% hot green (microwave)	nano Feo
Tannins Test	+	+	+	+	+	+	+	-
Carbohydrate Test	+	+	+	+	+	+	+	+
Glycosides Test	+	+	+	+	+	+	+	-
Phenols test	+	+	+	+	+	+	+	-
Resins Test	-	-	-	-	-	-	-	+
Flavonoids Test	+	+	+	-	+	+	+	+
Saponin Test	+	-	+	+	+	+	+	-
Alkaloid Test	+	+	+	+	+	+	+	+
Protien Test	-	-	-	-	-	-	-	-
Coumarins Test	+	+	+	+	-	-	-	-
Terpenes Test	-	-	-	-	-	-	-	-
Steroids Test	-	-	-	-	-	-	-	-
absence	-							
pH	7							
Temp.	25 C°							

Table(1)

Table (2) Aqeous Extract of *Z. moritiana*

TYPE OF ORGANISIM	Dry extract Mean ± SD Inhibition zone/mm		Green Extract Mean ± SD Inhibition zone/mm		Antibiotic control +	P value Between tested groups and control	P value Between tested groups only
	Hot	cold	Hot	Cold			
S. aureus GM+ve	C 12±0	B 15.33±1.5	C 11.33±1.2	C 11±3.6	A 30±1 (DA 10)	0.0001	0.002
S.epidermis GM +ve	B 14.33±2.1	C 11±1	D 9.33±1.2	B 14.33±4.1	A 18±2 (DO 10)	0.002	0.05
E. coli GM – ve	6.33±2.6	7.33±2.1	3±2.6	7±4.4	20±2 (C30)	0.001	0.09 N.SIG
Klebsiella species GM -ve	9.66	4	1.33	3.333	20±2 (MRP)	0.001	0.07 N.SIG
Candida albicans	5.66±1.1	9.66±0.5	10±1	9.66±1.2	14±1 (NYS)	0.04	0.06 N.SIG

*LSD test was used to calculate the significant differences between tested mean, the letters (A, B, C and D) LSD for represented the levels of significant, highly significant start from the letter (A) and decreasing with the last one.. **Similar letters mean there are no significant differences between tested mean p≤0.05 were considered significantly different**. Clindamycin 10 ug (DA 10)/ Doxycycline 10 ug (DO 10)

EXTRACTS TYPE OF ORGANISIM	DRY EXTRACT						Antibiotic + control	P value Between tested groups and control	P value Between tested groups only
	HOT			COLD					
Cons.	50%	70%	100%	50%	70%	100%			
Staphylococcus aureus GM+ve	15.33±1.3	12±1	16±0	9.33±1.3	15.667	12.3±1.3	30±1 (DA 10)	0.0001	0.09 nsig
Staphylococcus epidermis GM +ve	8.67±1.1	16.67±1.1	14.33±1.1	18.00±1.1	18.33±1.1	16.67±1.1	18±2 (DO 10)	0.06 nsig	0.012 nsig
Escherichia coli GM – ve	10.00±1	7.67±1.2	2.67±1.3	11.67±1.1	6.67±1.3	11.00±1.2	20±2 (C30)	0.001	0.32 nsig
Klebsiella species -GM -ve	10.00±1	8.00±0	12.00±0	11.33±1.3	11.67±1.2	12.00±1	20±2 (MRP)	0.002	0.211 nsig
Candida albicans	12.67±1.1	7.33±1.3	7.00±1	13.33±1.3	7.33±1.1	11.00±1	14±1 (NYS)	0.06 nisg	0.092 nsig

Table (3) Diameter of inhibition zone of ziziphus moratiana leaf extraction in methanol .

EXTRACTS TYPE OF ORGANISIM	GREEN EXTRCT						Antibiotic + control	P value Between tested groups and control	P value Between tested groups only
	HOT			COLD					
Cons.	50%	70%	100%	50%	70%	100%			
Staphylococcus aureus GM+ve	11±0	15.6±1.3	14.3±1.3	12.6±1.3	13.3±1.3	18±1.1	30±1 (DA 10)	0.0001	0.08 nsig
Staphylococcus epidermis GM +ve	E 8.67±1.1	C 15.00±1.1	B 19.33±1.1	C 14.33±1.1	D 12.33±1.1	A 24±1	B 18±2 (DO 10)	0.002	0.0031
Escherichia coli GM – ve	D 10.00±0	B 17.33±1.1	C 11.67±1.2	C 11.33±1.1	E 5.33±0.6	C 12±1	A 20±2 (C30)	0.001	0.05
Klebsiella species -GM -ve	6.67±1.3	8.67±1.1	7.33±1.1	6.67±1.1	6.67±1	9.67±1.3	A 20±2 (MRP)	0.002	0.74 NSIG
Candida albicans	11.67±1.3	14.33±1.3	7.67±1.3	9.33±1.3	7.00±0	11.67±1.3	14±1 (NYS)	0.05	0.63 NSIG

Table(4) Diameter of inhibition zone of ziziphus moratiana leaf extraction in methanol

EXTRACTS TYPE OF ORGANISIM	Inhibition zone of leaves extract Ethanol (10mg/ml) Green Extract						Antibiotic + control	P value Between tested groups and control	P value Between tested groups only
	HOT			COLD					
Cons.	50%	70%	100%	50%	70%	100%			
Staphylococcus aureus GM+ve	C 14.33±1.1	B 18±1	D 12±1	E 8±0	E 6±0	F 5±0	A 30±1 (DA 10)	0.0001	0.05
Staphylococcus epidermis GM +ve	C 12.33±1.2	A 19.67±1.3	B 16.67±1.3	C 13.3±1.1	C 12±0	C 11.67±1.2	A 18±2 (DO 10)	0.04	0.05
Escherichia coli GM – ve	11.67±0.9	11.33±1.1	10±0	6.67±1.2	0±0	12.3±1.3	A 20±2 (C30)	0.001	0.06 nsig
Klebsiella species -GM - ve	11±0	12.33±1.3	12.67±1.3	9.33±1.3	11.3±1.1	14±1.1	A 20±2 (MRP)	0.03	0.07 nsig
Candida albicans	C 8.33±1.1	B 12.67±1.3	C 7.67±1.3	E 2.67±0.3	D 6±0	E 2.33±1.3	A 14±1 (NYS)	0.0001	0.04

Table (5) Inhibition zone of leaves extract Ethanol (10mg/ml)

EXTRACTS TYPE OF ORGANISIM	Inhibition zone of leaves extract Ethanol (10mg/ml) Dry extract						Antibiotic + control	P value Between tested groups and control	P value Between tested groups only
	HOT			COLD					
Cons.	50%	70%	100%	50%	70%	100%			
Staphylococcus aureus GM+ve	14±1.1	16±0	13.3±1.1	15.3±1.3	14.3±1.1	13.3±1.1	A 30±1 (DA 10)	0.0001	0.082 nsig
Staphylococcus epidermis GM +ve	11.67±1.2	15±1.1	7.33±1.3	12.33	13.3±1	13±0	A 18±2 (DO 10)	0.05	0.09 nsig
Escherichia coli GM – ve	12±0	11.67±1.3	9±0	11±0	12.3±1.3	11±0	A 20±2 (C30)	0.05	0.073 nsig
Klebsiella species -GM - ve	10.67	10.67±1.2	10.33±1.3	11.33±1	11±0	11±0	A 20±2 (MRP)	0.042	0.12 nsig
Candida albicans	13±0	13±0	9.3±1.3	14±0	14.3±1.3	10.67±1.3	A 14±1 (NYS)	0.09 nsig	0.072 nsig

Table(6) Inhibition zone of leaves extract Ethanol (10mg/ml)

FTIR analysis : Tables 7,8,9 and 10 display the FTIR spectra of the leaf extracts of Z. *mauritiana*, revealing various naturally occurring compounds. Various natural compounds were generated by altering the solvents employed in the extraction of Z. *mauritiana* leaves, such as cold and hot ethanol and hot and cold water. The FTIR analysis yielded data regarding the functional groups present in the different extracts. The FTIR spectra of the leaf extracts exhibited distinct signals at specific wavelengths: 3345.57 cm-1 for the hot ethanol extract, 3265.53 cm-1 for the hot aqueous extract, 3360.09 cm-1 for the cold ethanol extract, and 3759.42 cm-1 for the cold aqueous extract. The phytochemical examination of the extracts revealed the presence of all the O-H and C=O functional groups, which are likely derived from polar natural compounds, as shown by a robust signal. Distinct signals were detected for the aromatic and aliphatic carbons at 2916.96 – 670.48, respectively. The phytochemical examination detected the existence of alkaloids, phenolics, carbohydrates, saponins, polyphenols, proteins, flavonoids, and amino acids, but they were present in varying proportions of transmittance (%). Table 7 compares the Ziziphus extracts using different solvents, showing that the cold aqueous extract has a greater total phenolic content (TPC).

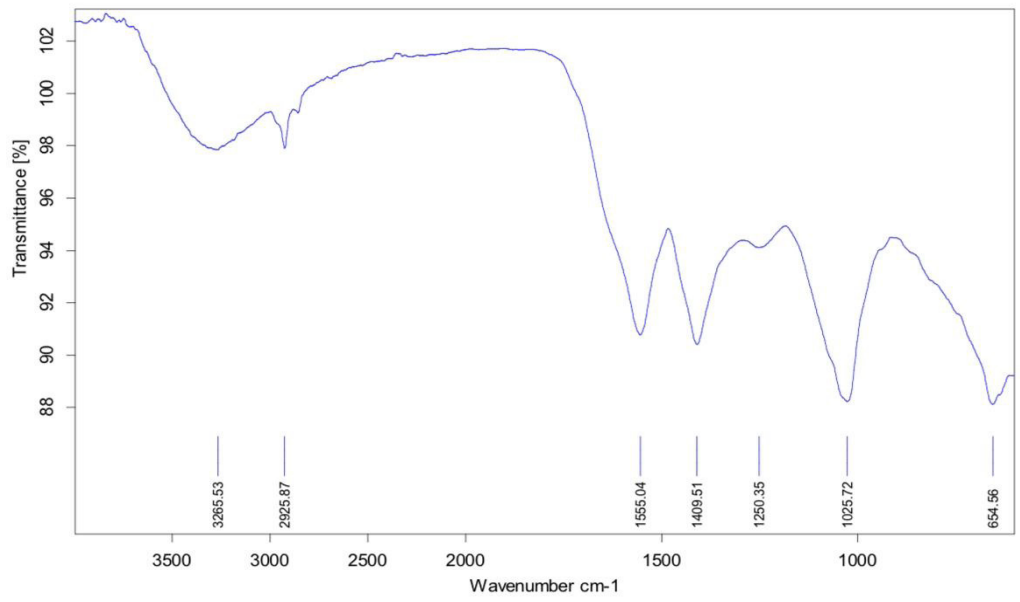

Figure (2): FTIR hot ethanolic dry extract

Table(7) hot ethanolic dry extract

No.	Frequency ranges (cm-1)	Frequency peak values (cm-1)	Vibration / bond	Specific functional group	Chemical compound
1	3570 - 3200	3345.57	H-bond OH- stretch	Alcohol and hydroxy compound	Hydroxy group
2	2935 – 2915	2916.96	C-H stretch	Alkanes	Aliphatic
3	3000 – 2850	2850.74	C-H stretch	Alkanes	Aliphatic
4	1715 – 1700	1709.85	C=O bend	Fatty acid group	Fatty acid
5	1680 – 1620	1655.45	C=C bend stretch	Alkene	aliphatic
6	1410 – 1310	1376.36	O-H bend	Alcohol hydroxy compound	Phenol or tertiary alcohol
7	1300 – 700	1204.56	C-C	Saturated Aliphatic (Alkene/Alkyl)	Methyne >CH-
8	1190 – 1130	1164.01	C-N stretch	Secondary amino	Secondary amin
9	1050 – 990	1040.11	P-O-C stretch	Phosphorus -oxy compound	Aliphatic phosphate
10	1210 – 995	994.52	COO-	Carboxyl group	carboxyl
11	1100 – 900	923.34	Cis-CH-CH	Common inorganas ion	Stretch out of plant
12	890 – 820	839.69	C-O-O stretch	Alcohol and hydroxy compound	alcohol
13	890 – 820	839.64	C-O-O stretch	Ether and oxy compound	Alcohol
14	680 – 610	670.48	C-H bend	Alcohol and hydroxy compound	alcohol

GC-MS analysis: The analysis of five Ziziphus extracts reveals the existence of around 10 to 25 components, which include aromatic rings, alkenes, indole rings, carboxylic acids, esters, nitro compounds, aldehydes, ketones, and phenolic compounds (refer to tale 11, 12, 13, 14 and 15). The overall area has varied with each form of extraction. The aqueous extract, which was hot and dry, exhibited a total area percentage of 99.99%. The area percentages of its peaks ranged from 12.75% to 2.36%. The concentration was detected by 1,4-Butanediol with a retention period of 5.041 minutes. The aqueous total area was cold and dry, with a percentage of 100.01%. The area percent-

age ranges from 7.47% to 21.58%. The highest concentration was detected in cis-aconitic anhydride with a retention period of 5.249 minutes. The entire area of the cold, dry ethanolic extract was 100%. The area percentage ranges from 1.54% to 59.22%. Diglycerol's highest concentration was discovered at a retention period of 5.543 minutes. The overall area of the hot, dry ethanolic extract was 99.98%. The area percentage ranges from 1.86% to 21.61%. The highest concentration was seen in Glycerin at a retention period of 6.131 minutes, while the total area of the hot green ethanolic extract was 99.98%. Although it displayed a range of values, the compound with the highest area percentage was 21.61% in 3,5-Heptadien-2-one, 6-methyl-, at a reaction time of 5.917. The compound with the lowest area percentage was 1.86%.

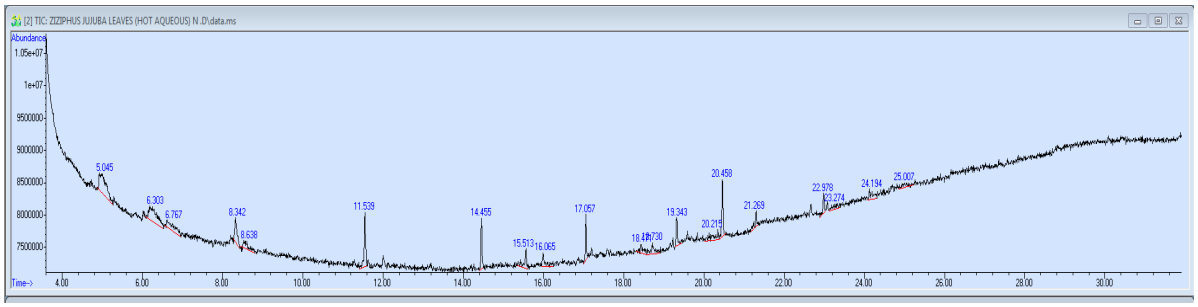

Figure (3): GC MS of *hot aqueous dry extract*

Table(8) hot aqueous dry extract

Peak No.	Compound	Retention time(min)	Area %	Formula	Molecular Weight g/mol
1	1,4-Butanediol	5.041	12.75	C4H10O2	90.12
2	3-Chloro-N-[2-methyl-4(3H)-oxo-3-quinazolinyl]-2-thianaphthenecarboxamide	6.305	10.86	C18H12ClN3O2S	369.83
3	cis-Aconitic anhydride	6.763	28.56	C6H4O5	156.09
4	tert-Butyldimethyl(3-(methylthio)propoxy)silane	11.541	7.45	$Br(CH_2)_3OSi(CH_3)_2C(CH_3)_3$	253.25
5	Bis(2-methylpropyl) 4-methylbenzen e-1,2-dicarboxylate	11.541	7.45	C17H24O4	292.38
6	Methyl 2-ethyl-4-(trimethylsilyloxy)hexylphthalate	11.541	7.45	$C_{16}H_{20}O_6$	308.33
7	Disilathiane, hexamethyl-	14.458	6.15	C6H18SSi2	178.448
8	Thiophene, 2-(trimethylsilyl)-5- trimethylsilyl)ethynyl]-	14.458	6.15	C9H12SSI	180.34

Peak No.	Compound	Retention time(min)	Area %	Formula	Molecular Weight g/mol
9	cis-4-Ethoxy-3-methoxy-.beta.-methyl-.beta.-nitrostyrene	14.458	6.15	C10H11NO3	193.204
10	Ethanedicarboxamide, N-allyl-N' ,5-dimethylphenyl)-	15.514	15.95	C13H16N2O2	232.28
11	Silane, dimethyl(4-acetylphenoxy)propoxy-	17.055	5.02	C13H20OSI	252.38
12	Indolo[2,3-b]quinoxaline, 1-fluoro	17.055	5.02	C14H9N3	219.24
13	4,6-Difluoro-2-(4-methoxyphenylami no)pyrimidine	17.055	5.02	C5H4F2N2S	162.16
14	Hexadecanoic acid, methyl ester	20.456	7.24	C17H34O2	270.4507
15	Pentadecanoic acid, methyl ester	20.456	7.24	C16H32O2	256.42
16	2,2'-(1,4-Piperazinediyl) bis[N-(4- methoxyphenyl) succinimide]	22.975	2.88	C26H28N4O6	492.5

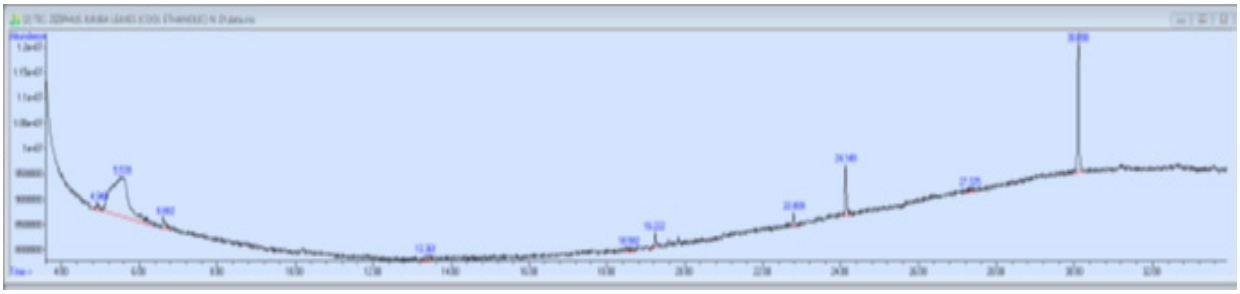

Figure (4): cool ethanolic dry extract

Table(9) cool ethanolic dry extract

Peak No.	Compound	Retention time(min)	Area %	Formula	Molecular Weight g/mol
1	1,2-Hydrazinedicarboxylic acid, diethyl ester	4.946	1.64	C3H8N2O2	104.1078
2	Carbamic acid, (2-chloroethylidene)bis-, diethyl ester	4.946	1.64	C12H24N2O4	260.330
3	Diglycerol	5.543	59.22	C6H14O5	166.17
4	Glycerin	5.543	59.22	C3H8O3	92.09
5	Oxirane, (ethoxymethyl)-	6.660	2.62	C5H10O2	102.1317
6	Hydrazinecarboxylic acid, ethyl ester	6.660	2.62	$C_3H_8N_2O_2$	104.1078
7	Urea	13.359	2.47	C7H2OSI2	204.4175
8	Ethanedicarboxamide, N-allyl-N'-(2 ,5-dimethylphenyl)-	13.359	6.86	C13H16N2O2	232.28
9	cis-Aconitic anhydride	18.578	15.11	C6H4O5	156.09
10	Squalene	30.098	18.94	C30H5O	410.7180

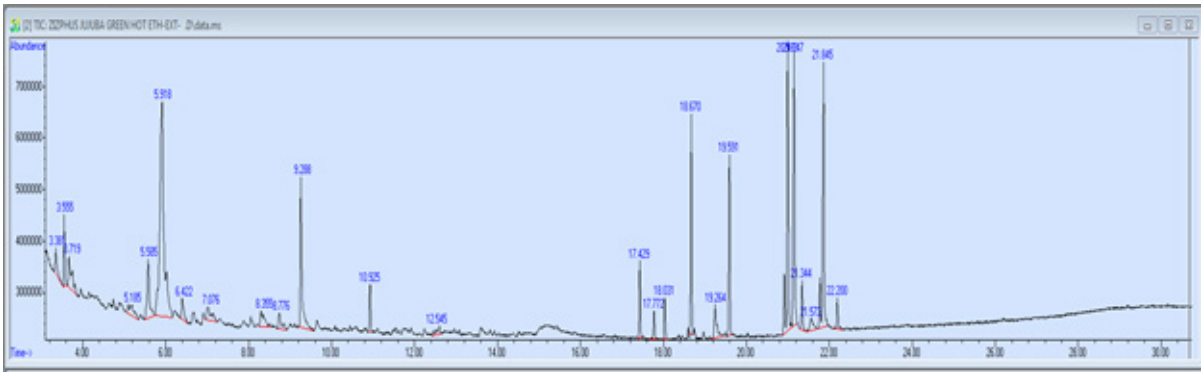

Figure (5): hot ethanolic dry extract

Table(10) hot ethanolic dry extract

Peak No.	Compound	Retention time(min)	Area %	Formula	Molecular Weight g/mol
1	1,2-Hydrazinedicarboxylic acid, diethyl ester	4.043	4.94	C3H8N2O2	104.1078
2	Carbamic acid, (2-chloroethylidene) bis-, diethyl ester	4.043	4.94	$C_8H_{15}ClN_2O_4$	238.66900
3	Oxirane, (ethoxymethyl)-	4.043	4.94	$C_8H_{16}O_3$	160.21100
4	1,2,3,4-Butanetetrol, [S-(R*,R*)]-	5.041	5.02	C3H7CLO2	110.54
5	Glycerin	6.131	21.61	C3H8O3	92.0938
6	cis-Aconitic anhydride	8.391	23.24	C6H4O5	156.09
7	2,2'-(1,4-Piperazinediyl)bis[N-(4-methoxyphenyl)succinimide]	8.391	3.13	$C_{26}H_{28}N_4O_6$	492.5
8	4-Amino-5-imidazole carboxamide,N, N,O- tris(trimethylsilyl)-	9.871	4.28	∘ C4H7CIN4O	162.58
9	2,4,6(1H,3H,5H)-Pyrimidinetrione, 5-ethyl-5-(1-methylethyl)-1,3-bis(trimethylsilyl)-	9.871	4.28	∘C17H34N2O-3Si2	370.6
10	3,5-Diisopropoxy-1,1,1,7,7,7-hexa-methyl-3,5-bis(trimethylsiloxy)tetr asiloxane	9.871	4.28	∘ C18H50O-7Si6	547.1
11	1,2,2,3,4-Butanepentacarbonitrile	10.303	2.13	∘ $C_9H_5N_5$	183.17
12	Ethanedicarboxamide, N-allyl-N'-(2,5-dimethylphenyl)-	10.624	4.35	$C_{13}H_{16}N_2O_2$	232.28
13	Urea	10.642	2.49	C6H4O5	156.09
14	Cycloheptasiloxane, tetradecamethy	12.225	3.32	C14H42O7Si7	519.07

Peak No.	Compound	Retention time(min)	Area %	Formula	Molecular Weight g/mol
15	Pentasiloxane, dodecamethyl-	12.225	3.32	C12H36O4Si5	384.8393
16	3-Chloro-N-[2-methyl-4(3H)-oxo-3-quinazolinyl]-2-thianaphthenecarboxamide	12.874	2.02	C6H9ClO2	148.59
17	Cyclooctasiloxane, hexadecamethyl-	15.756	2.87	C16H48O8Si8	593.2
18	Hexasiloxane, tetradecamethyl-	15.756	2.87	$C_{14}H_{42}O_5Si_6$	458.9933
19	2,2'-(1,4-Piperazinediyl)bis[N-(4-methoxyphenyl)succinimide]	18.310	1.94	C10H16Br-2N2O2	356.054
20	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	18.310	1.94	$C_{13}H_{39}O_5Si_6$	443.96
21	Cyclononasiloxane, octadecamethyl-	18.310	1.94	C18H54O-9Si9	667.38546
22	4(1H)-Pyrimidinone, 2-(ethylthio)-	22.810	3.75	$C_5H_6N_2OS$	*142.179*
23	Spirohexan-4-one, 5-chloro-6,6-dimethyl	22.810	3.75	$C_8H_{11}ClO$	158.62
24	1,2,5-Oxadiazole	14.596	1.86	$C_2H_2N_2O$	70.0501
25	Squalene	30.115	25.41	C30H5O	410.7

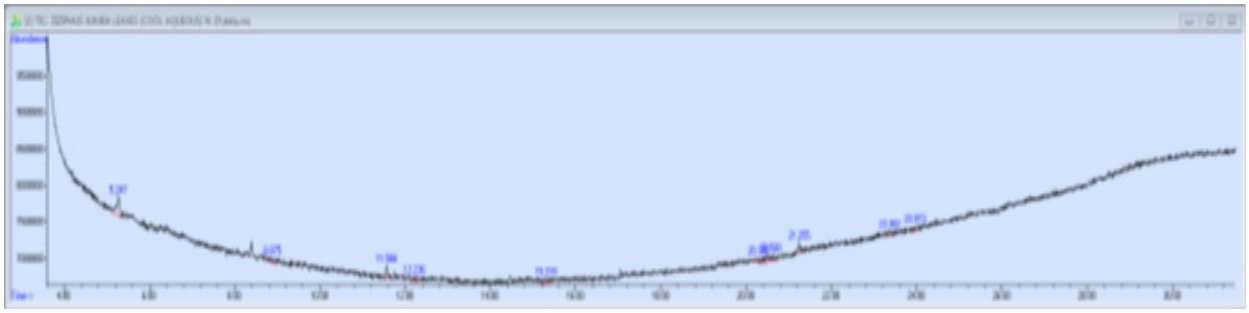

Figure (6): cool aqueous dry extract

Table(11) cool aqueous dry extract

Peak No.	Compound	Retention time(min)	Area %	Formula	Molecular Weight g/mol
1	cis-Aconitic anhydride	5.249	68.058	C6H4O5	156.09
2	3-Butyn-1-ol	5.249	21.58	C_4H_6O	70.09
3	Ethanedicarboxamide, N-allyl-N'-(2,5-dimethylphenyl)-	8.875	45.25	$C_{13}H_{16}N_2O_2$	232.28
4	3-Chloro-N-[2-methyl-4(3H)-oxo-3-quinazolinyl]-2-thianaphthenecarbox amide	8.875	28.24	C6H4O5	156.093
5	Pyrimidine, 4,6-dimethoxy-5-nitro	11.567	25.01	$C_6H_7N_3$	185.137

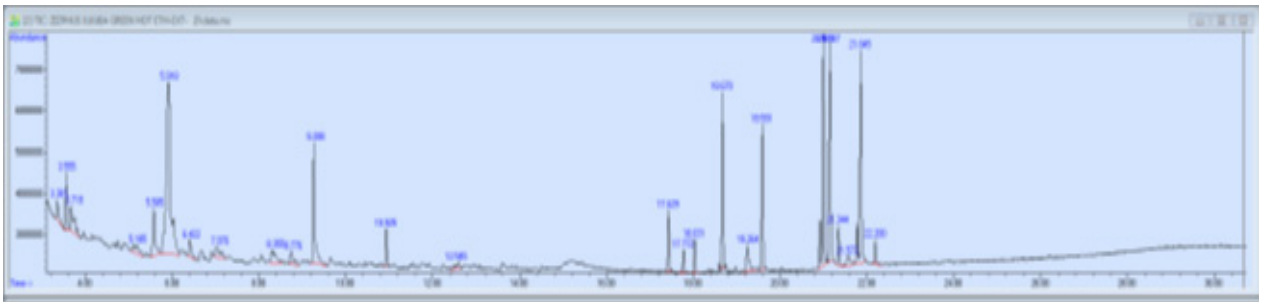

Figure (7): *hot green ethanolic extract*

Table (12) hot green ethanolic extract

Peak No.	Compound	Retention time(min)	Area %	Formula	Molecular Weight
1	Oxime-, methoxy-phenyl	3.554	1.7	C8H9NO2	151.16
2	Adenosine, 2-methyl-	3.719	2.44	C10H13N5O4	267.24
3	Urethane	5.181	1.33	C4H8O	72.1057
4	Tris(tert-butyldimethylsilyloxy)arsane	5.588	3.29	C18H45A-so3Si3	468.7
5	Tetrasiloxane, decamethyl-	5.588	3.29	C10H30O3Si4	310.6854
6	Cyclotrisiloxane, hexamethyl-	5.588	3.29	C6H18O3Si3	222.46
7	3,5-Heptadien-2-one, 6-methyl-	5.917	22.28	C8H12O	124.1803
8	Pyrido[3,2-d]pyrimidin-4(3H)-one, 3-hydroxy-2-methyl-	6.419	1.17	C8H7N3O2	177.16
9	4-Ethylbenzamide	6.419	1.17	C9H11NO	149.19
10	3-Bromo-3-buten-1-ol	6.419	1.17	C4H7BrO	151
11	1,2-Hydrazinedicarboxamide	7.077	1.44	CH5N3O	75.0699
12	Oxiranemethanol, (R)-	7.077	1.44	C3H6O2	74.0785
13	Azetidin-2-one 3,3-dimethyl-4-mino-ethyl)-	7.077	1.44	C7H14N2O	142.20
14	Chloroacetylamine, N-[2-n-octyl]-	8.358	1.61	C8H14N4OS	214.288
15	Benzofuran, 2,3-dihydro-	8.358	1.61	C8H8O	120.1485
16	Catecholborane	8.358	1.61	C6H4O2BH	119.92
17	2-Amino-4-dimethylaminomethylene-pentanedinitrile	8.773	1.14	C5H11NO2S	149.211
18	2-Dimethylaminomethyl-4-chloro-1-naphthol	8.773	1.14	C10H7ClO	178.61
19	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-	8.773	1.14	C13H22Osi2	250.48

Peak No.	Compound	Retention time(min)	Area %	Formula	Molecular Weight
20	8-Azabicyclo[3.2.1]octan-3-ol, 8-methyl-, endo-	9.293	6.41	C8H15NO	141.2108
21	1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta[c]pyran-1-yl) ethano Ne	10.929	1.44	C10H16O2	168.2328
22	N-[[2-Oxo-1,2'-bi(tricyclo[3.3.1.1[3,7]]decan)-4'-yl] methyl]acetamid	10.929	1.44	C7H8N2O2S	184.22
23	Isophthalic acid, 2,6-dimethylnon-1-en-3-yn-5-yl propyl ester	10.929	1.44	C22H28O4	356.4553
24	Butanal, 3-hydroxy-	10.929	1.44	C4H8O2	88.1051
25	2(3H)-Furanone, dihydro-4-hydroxy-	10.929	1.44	C4H6O3	102.09
26	L-Alanine, methyl ester	10.929	1.44	C4H10CINO2	139.85
27	9-Octadecyne	17.429	4.36	C18H36	252.5
28	trans-2-Dodecen-1-ol	17.429	2.14	C12H24O	184.32
29	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	17.775	0.97	C20H40O	296.5310
30	Cyclopentane, 1,2-dimethyl-3-(1-methylethenyl)-	17.775	0.97	C10H18	138.2499
31	Hexadecanoic acid, methyl ester	18.666	6.72	C17H34O2	270.4507
32	n-Hexadecanoic acid	19.264	1.96	C16H32O2	256.4241
33	Hexadecanoic acid, ethyl ester	19.593	5.29	C18H36O2	284.4772
34	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	20.986	11.52	C19H32O2	292.4562
35	Phytol	21.150	9.69	C20H40	296.5
36	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, [1S-(1.alpha.,2.beta.,5.beta.)]-	21.150	9.69	C10H20O	156.2652
37	Benzyl alcohol, p-hydroxy-.alpha.-[(methylamino)methyl]-	21.575	1.05	C9H14CINO2	203.66
38	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	21.843	10.66	C18H30O2	278.4
39	Ethyl 9,12,15-octadecatrienoate	21.843	10.66	C20H34O2	306.4828
40	Octadecanoic acid, ethyl ester	22.198	1.14	C20H40O2	312.5304
41					

DISCUSSION

Microwave-assisted extraction (MAE) is a method that harnesses the capabilities of microwaves to induce molecular motion in liquids, hence facilitating the effective extraction of desired constituents. MAE provides advantages over conventional extraction techniques, including decreased extraction durations, diminished solvent expenses, and enhanced automation. The process of MAE facilitates uniform heating of both the solvent and the material, leading to an enhanced extraction yield of chemicals. Microwave-assisted extraction (MAE) is a method that harnesses the capabilities of microwaves to induce molecular motion in liquids, hence facilitating the effective extraction of desired constituents. MAE provides advantages over conventional extraction techniques, including decreased extraction durations, diminished solvent expenses, and enhanced automation. According to Sun et al. (2020), the application of MAE leads to uniform heating of the solvent and the material, enhancing the extraction yield of chemicals. Ethanol and methanol cannot dissolve polysaccharides, tannins, gums, and waxes despite their effectiveness as solvents for alkaloids, flavonoids, terpenes, glycosides, and coloring chemicals. When selecting a solvent for the extraction procedure, it is crucial to thoroughly assess multiple parameters, such as the solvent's capacity to dissolve the targeted chemicals, its boiling point, reactivity, viscosity, recovery rate, vapor pressure, safety profile, toxicity, and cost.

The solvent selection is contingent upon the characteristics of the compounds under extraction and the desired objectives of the extraction procedure (Proestos & Komatis, 2008). The initial analysis of phytochemicals revealed the presence of many bioactive constituents, including saponins, tannins, alkaloids, phenolic compounds, and terpenoids. The existence of these constituents serves as a sign that this botanical specimen possesses certain therapeutic attributes. The results of the present study are consistent with prior research since Najafi (2013) documented the detection of saponins, phenolic compounds, tannins, and glycosides in the leaves of Z. *mauritiana*. According to Parmar et al. (2012), the leaves of Z. *mauritiana* possess glycosides, saponins, phenols, lignins, and

tannins. Saponin-rich plants exhibit anti-inflammatory properties and enhance immune system function. According to Mainasara et al. (2012), tannins are a class of antibacterial chemicals that can harm the bacteria's cell wall. Biapa et al. (2007) have identified phenolic substances, alkaloids, flavonoids, tannins, saponins, and glycosides as potent antioxidants that effectively regulate oxidative stress-related illnesses.

The zone of inhibitions observed with the methanol leaf extract of Z. *mauritiana* exhibited variability. The maximum sensitivity was observed in Bacillus cereus ATCC 10876 (Gram-positive) and Proteus vulgaris (Gram-negative). Several research studies have reported the antibacterial properties of Z. *mauritiana* leaves against various bacterial strains. Najafi (2013) highlighted the significant activity of methanol leaf extracts of Z. *mauritiana* against S. aureus and E. coli. According to Ashraf et al. (2015), the methanol leaf extract of Z. *mauritiana* exhibits strong antibacterial properties against E. coli, B. subtilis, and S. aureus. However, the present investigation did not observe significant antibacterial activity in other clinical infections, including multidrug-resistant (MDR) and non-multidrug-resistant (non-MDR) isolates. Furthermore, a publication revealed that the leaves of Z. *mauritiana* do not exhibit any antibacterial properties against some bacterial strains.

According to Mainasara et al. (2012), the antimicrobial activity of methanol and ethanol extracts against *P. aeruginosa, S. typhi*, E. coli, *S. aureus*, and *S. pyrogenes* was shown to be insignificant at a concentration of 120 mg/ml. Therefore, further research is necessary to investigate this botanical extract's mechanism and mode of action.

Most isolated compounds demonstrated diverse actions, including antibacterial, antifungal, anti-inflammatory, anti-histamine, and other properties. 1,4-Butanediol has been discovered to stimulate the release of growth hormones under specific conditions (Vance et al. et al., 1995). Cis-aconitic anhydride has been found to possess antiviral activity (Reményi et al., 2003). Hexadecanoic acid and methyl ester have demonstrated antifungal activity (Abubacker et al., 2013). Diglycerol and its derivatives have exhibited significant antifungal properties against various plant pathogenic fungi (Shimazaki A. et al., 2016). Glycerin

has demonstrated antibacterial properties (Owain et al., 2020) and anti-irritant and anti-inflammatory effects (Szél, E. et al., 2015). Additionally, it has been found to promote wound healing (Bialik-Wąs, K. et al., 2021). Also, Heptadien has been discovered to possess anticancer and antifungal properties (Sun J. et al., 2020).

CONCLUSION

Medicinal advancements often draw inspiration from natural products. The leaves of Z. mauritiana contain a significant amount of phytochemical constituents. The components above possess antibacterial, anti-inflammatory, and antioxidant properties. Various bioactive phytochemicals have the potential to be extracted and refined, resulting in significant therapeutic properties such as antibacterial, antioxidant, anti-inflammatory, and other beneficial effects against a range of diseases.

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