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Research Article

GC- MS Analysis and Antimicrobial Activity of *Typha domingensis* Pers. Aerial Parts.

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ABSTRACT

Typha domingensis Pers. is an aquatic Typhaceae family member. GC/MS examination of saponifiable matter of *Typha domingensis* Pers. aerial parts identified twenty-three known fatty acids, including 9,12-Octadecadienoic acid, methyl ester, (*E*,*E*), and 9-Octadecenoic acid (*Z*)-, methyl ester as the major constituents, representing peak area percentage of 11.623 % and 9.502 %, respectively. The examined plant methanol extract revealed a high total phenolic (158.3939 ± 1.867 µg gallic acid/mg extract) and flavonoid (40.5813 ± 0.4098 µg rutin /mg extract) contents. In this study we investigated the antibacterial activity of *T. domingensis* Pers. total methanol extract of aerial parts (TDME) and its successive fractions. Values of MIC were detected against several strains of bacteria. It has been found that TDME demonstrated inhibitory effects against Gram-positive bacteria (*Bacillus subtilis*) with MIC value 4 µ/mL equal to MIC value of the control (ampicillin). Among the tested fractions, the ethyl acetate fraction (TDEF) showed notable antibacterial activity against both *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria with MIC values equal to that of the control. Furthermore, TDEF showed MIC value against *B. subtilis* lower than the MIC value of the control. The antifungal efficacy of the extracts was also investigated against *Candida albicans* and *Aspergillus flavus* compared to positive control (clotrimazole). All the tested fractions had modest antifungal effect.

Keywords: *Typha domingensis* Pers., GC/MS, Antimicrobial activity.

1. INTRODUCTION

Typha domingensis Pers., usually referred to as southern cattail, is an aquatic plant that flourishes in wetland habitats across the globe. It is a member of the Typhaceae family, which encompasses 30 species within the genus Typha[1]. In Turkey, the female parts of Typha species are traditionally used as a haemolytic agent[2]. The pollen grains of *T. domingensis* Pers. has several medicinal properties, including astringent, diuretic and desiccant characters[3]. *T. domingensis* Pers. is not only used as a traditional medicine, but also serves as a food source in some regions. In addition to methanolic extract of the leaves of *T. domingensis* Pers., other extracts of Typha species such as *T. elephantina*, *T. angustifolia*, and *T. latifolia* have demonstrated antimycobacterial properties[4,5] One major issue facing modern healthcare is the rapidly increasing prevalence of multidrug-resistant microorganisms (MDR). These antibiotic-resistant pathogens are a major factor contributing to treatment failures and increased mortality rates[6].

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Efforts are coordinated to implement antimicrobial stewardship, enhance infection prevention and control measures, and develop new antibiotics are urgently needed to address this global public health threat. The development of novel antibacterial agents is critically needed, that can effectively combat drug resistance and efficiently treat infectious diseases[7]. The growing prevalence of antifungal resistance is another pressing concern in the healthcare sector, necessitating the development of innovative antifungal agents. Opportunistic fungi, such as *Candida albicans* and *Aspergillus fumigatus*, pose a significant threat as they can cause invasive fungal infections in various organs of the human body[8]. Numerous plants exhibit antibacterial and antifungal effects comparable to standard antibiotics, making them promising alternatives in the fight against microbial infections[9].

These days, GC-MS is an essential technique for the thorough profiling of secondary metabolites in a variety of organisms, including plants and non-plant species, identification and quantification of a diverse array of secondary metabolites[10].

The beneficial effects of medicinal plants on health are caused mainly by phenolic content. If they are used, there may be a decreased chance of diseases like cancer and heart diseases. Phenolic and flavonoid compounds pose a variety of uses, including antimicrobial, antithrombotic, and antitumor effects[11].

This study aims to explore the antimicrobial potential of the methanol extract and different fractions of *T. domingensis* Pers. aerial parts, known for their traditional medicinal uses. Focusing on their total phenolic and flavonoid contents, the research seeks to identify natural alternatives to conventional antibiotics, addressing both bacterial and fungal resistance issues that pose a critical global health challenge.

Fatty acids are essential molecules in biological systems and have several important biological functions. Studies have indicated that imbalances in fatty acids are associated with a wide variety of diseases, such as inflammation, cardiovascular disease, tumorigenesis and Alzheimer's disease[12]. So, in this research we focus on GC/MS analysis of the fatty acid methyl esters fraction of *T. domingensis* Pers. aerial parts to identify possible phytochemicals present in this fraction.

2. MATERIALS AND METHODS

2.1. General

Analytical grade solvents were used in this experiment. A GC/MS Finnigan mat. SSQ 7000, Trace GC 200 (thermo) GC mode, USA was used. FluoStar Omega microplate reader.

2.2. Plant Material

In January 2021, aerial parts of T. domingensis Pers. were collected in Damietta Governorate from Al-Rakabeyah, Kafr El Battikh. Prof. Dr. Dalia Ahmed, Faculty of Science, Tanta University kindly identified it. A voucher sample number PGG-TD120 was kept at the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Tanta University.

2.3. Preparation of unsaponifiable and saponifiable matter.

Methanol was used in cold maceration to extract the plant powder (2 kg) until it was fully extracted. Under low pressure, the entire methanol extract was evaporated, producing a green residue weighing 170 g (TDME). The residue from the methanol extract (150 g) was suspended in 750 mL of 50% aqueous methanol and then fractionated using different solvents. After evaporation, the residues were weighed to produce 20.95 g, 7.21 g, 10.85 g and 28.82 g of *n*-hexane fraction (TDHF), methylene chloride fraction (TDMF), ethyl acetate fraction (TDEF), and *n*-butanol fraction (TDBF), respectively.

TDHF was used to prepare the saponifiable (TDHF-SAP) and unsaponifiable matter (TDHF-USM) [13]. 180 mL of alcoholic KOH (10%) was added to (12 g) of TDHF, which was heated for six hours using a reflux condenser and boiling water bath. The saponified liquid was distilled to remove the majority of the alcohol. After being diluted with 120 mL of water, the aqueous liquid was extracted using 120 mL of ether until exhaustion. The TDHF-USM was obtained by distilling combined ethereal extracts under low pressure after they were washed with D.W. and dried over anhydrous Na_2SO_4.

After TDHF-USM extraction, concentrated HCl was used to acidify the alkaline aqueous solutions, and ether was used to extract the released fatty acids. To obtain the fatty acid fraction, the ether extracts were dehydrated over anhydrous Na_2SO_4 and distilled after being washed with D.W. TDHF-SAP was methylated according to Vogel's method[14].

One µL of methyl ester residue (1 mg/mL) was mixed in analytical grade ether for GC/MS analysis.

2.4. GC/MS analysis:

Before GC/MS analysis, sample equilibrium was thoroughly processed GC/MS Finnigan mat. SSQ 7000, Trace GC 200 (thermo) GC mode, USA at 28°C. The applied column (Elite-5MS) was described as by 30 m of length with inner diameter at 0.25 mm while the thickness film was 0.25 µm. Split mode was implemented for injections with a split ratio of 20:1 under conditions of: injector temp. 280°C, column oven temp. 80°C for 4 min, ramp 8°C/min to 230°C, hold 1 min, ramp 8°C/min to 250°C, hold 1 min, ramp 8°C/min to 280°C, hold 3 min, when the flow rate of carrier gas (He) was 1 mL/min. Transfer line temp. was set at 280°C and ion source temp. adjusted at 180°C. Electron ionization mode (EI, 70 eV.) with scan range of *m/z* 50–650 was used.

The identification of compounds was achieved by comparing their retention times and mass spectra with those of the available reference in GC/MS library (MAINLIB and replib) which had been tested under similar conditions. AMDIS software was involved in identification.

2.5. Total Phenolic content.

Using Attard's technique, the total phenolic content of TDME and TDEF was ascertained[15]. A stock solution of 2 mg/ml of gallic acid in methanol was prepared to create the calibration curve. Dilutions of 25, 50, 100, 200, 400 µg/ml were used. Each sample was made with methanol at a 1 mg/mL concentration. The process was as follows:

In a 96-well microplate, incorporate 10µL of the sample or standard into 100µL of diluted Folin Ciocalteu reagent. After adding 80µL of 1M Na_2CO_3, the mixture was allowed to sit at room temperature (25°C) in the dark for 20 minutes. Following the incubation period, the ultimate color of the blue complex was measured at 630 nm. The findings were recorded as means ± SD.

2.6. Total flavonoid content.

The total flavonoid content of TDME and TDEF was found out, utilizing the technique of aluminum chloride[16], with slight adjustments to be made in microplates. To obtain the calibration curve, standard rutin was prepared as a stock solution in methanol at a dose of 20000 µg/ mL. from which the dilutions listed below were made: 500, 250, 125, 62.5, 31.25, and 15.625 µg/mL. Each sample was made with methanol at 1 mg/mL concentration. In brief, a 96-well microplate was filled with 15 µL of sample or standard, 175 µL of methanol, and 30 µL of 1.25% $AlCl_3$. Following a 5-minute incubation period, 30 µL of 0.125 M sodium acetate was added. The resulting yellow color from the incubation period was measured at 420 nm. The findings were recorded as means ± SD.

2.7. Antimicrobial activity

All tested organisms came from American type tissue culture collection (ATCC). Ampicillin, Clotrimazole and DMSO were obtained from Sigma Chemical Co., United States.

The microdilution susceptibility test was used to evaluate the antibacterial and antifungal activity in Müller-Hinton broth (Oxoid) and sabouraud liquid medium (Oxoid), respectively[17]. Stock solutions for the investigated fractions, ampicillin and clotrimazole were prepared in DMSO with a 1000 mg/mL concentration. To create two-fold serial dilutions, each stock solution was diluted with standard technique. In each well of a 96-well microliter plate, ten milliliters of the broth, which contained roughly 10^6 colony-forming units per milliliter of tested bacteria were added. For 24 hours at 36°C to test antibacterial activity and 48 hours at 36°C to test antifungal activity, the sealed microplates were incubated in a humid atmosphere. The lowest concentrations of the substance that did not exhibit any turbidity were determined to be the minimum inhibitory concentrations (MICs) values after the incubation period. Parallel to the tested fractions, identical procedures were applied to control studies using DMSO and an uninoculated medium.

3. RESULTS AND DISCUSSION

3.1. Examination of the methylated TDHF-SAP fraction by GC/MS :(Figure 1)

GC/MS was used to identify phytochemical compounds in methylated TDHF-SAP fraction. The total percent of identified fatty acids is 46% as twenty-three compounds were identified. Different saturated and unsaturated compounds were detected. These compounds are demonstrated in **Table 1 and Figure 1** with their molecular mass. Saturated compounds composed 39.2% relative to the total identified compounds, while unsaturated formed 60.8%. Compounds (**12**), (**13**), and (**4**) were the

major compounds with the largest peak area percentages representing 11.623 %, 9.502 %, and 6.596 %, respectively of total identified fatty acids of this fraction.

The mass spectrum 9,12-Octadecadienoic acid, methyl ester, (*E*,*E*) (11.623%) is written by *m/z* 294 originating from the molecular ion of $C_{19}H_{34}O_2^+$ which is formed when exposed to 70 eV. of energy. The molecular ion of $C_{19}H_{34}O_2^+$ undergoes McLafferty reorganization and fragmentation (beta to double bond) to produce fragment (the base beak) by *m/z* 67 originating from $C_5H_7^+$ according to previous literature[18]. This compound can make another fragmentation pattern according to another literature to produce 3-penteyene (*m/z* =67), butyne ion (*m/z* =55) and propyne ion (*m/z* =41), these peaks are the major peaks in our mass spectrum[19]. The mass spectrum and fragmentation is displayed in **Figure 2.**

The mass spectrum of 9-Octadecenoic acid (*Z*)-, methyl ester (9.502%) is written by *m/z* 296 originating from the molecular ion of $C_{19}H_{36}O_2^+$ which is formed when exposed by 70 eV energy. Molecular ion of $C_{19}H_{36}O_2^+$ can experience fragmentation by releasing CH_3OH and it produces the peak within *m/z* 264 originating from $C_{18}H_{32}O^+$. The peak with *m/z* 111 originating from $C_7H_{11}O^+$ releases C_2H_2O and produces *m/z* 69 originating from $C_5H_9O^+$; while the base peak originates from $C_4H_7^+$ with *m/z* 5518 .The pattern of fragmentation of 9-Octadecenoic acid (Z)-, methyl ester can be seen in **Figure 3**.The spectrum of fragmentation mass of Tridecanoic acid [18], methyl ester (6.596%) is written by *m/z* 228 of molecular ion of $C_{14}H_{28}O_2^+$ resulted from Tridecanoic acid, methyl ester when it is exposed with 70 eV. of energy. Molecular ion of $C_{14}H_{28}O_2^+$ undergoes McLafferty reorganization and produce fragment by *m/z* 74 originating from $C_3H_6O_2^+$. The mass spectrum and fragmentation is displayed in **Figure 4**.

Major compounds detected in this fraction were reported to possess important biological activities and proven medicinal uses. For instance, The major compound 9,12-Octadecadienoic acid, methyl ester, (*E*,*E*) (11.623%) is reported to have anti-inflammatory , insectifuge, antiarthritic and nematicide activity [20]. 9-Octadecenoic acid (*Z*)-, methyl ester (9.502%) had also reported anti-inflammatory and insectifuge activity.[20] Tridecanoic acid, methyl ester (6.596%) had reported antibacterial and anti-enteric activity[21].

The three major compounds were not previously reported in *T. domingensis* Pers. but according to previous studies some related fatty acids had been identified in the same plant by GC/Mass analysis as 8, 11-Octadecadienoic acid (9.38 %), 8-Octadecenoic acid (1.06 %), Tridecanoic acid (0.38%) and 9-Octadecenoic acid (12.07%)[22].

3.2. TDME total phenolic content

Considering the findings, the total phenolic content of TDME is 158.3939 ± 1.867 expressed as µg gallic acid /mg extract. Results also can be presented as 12.1841± 1.867 expressed as µg gallic acid/mg dried weight of plant material.

3.3. TDME total flavonoid content

Considering the findings, the total flavonoid content of TDME is 40.5813 ± 0.4098 expressed as µg rutin/mg extract. Results also can be presented as 3.1216± 0.4098 expressed as µg rutin/mg dried weight of plant material.

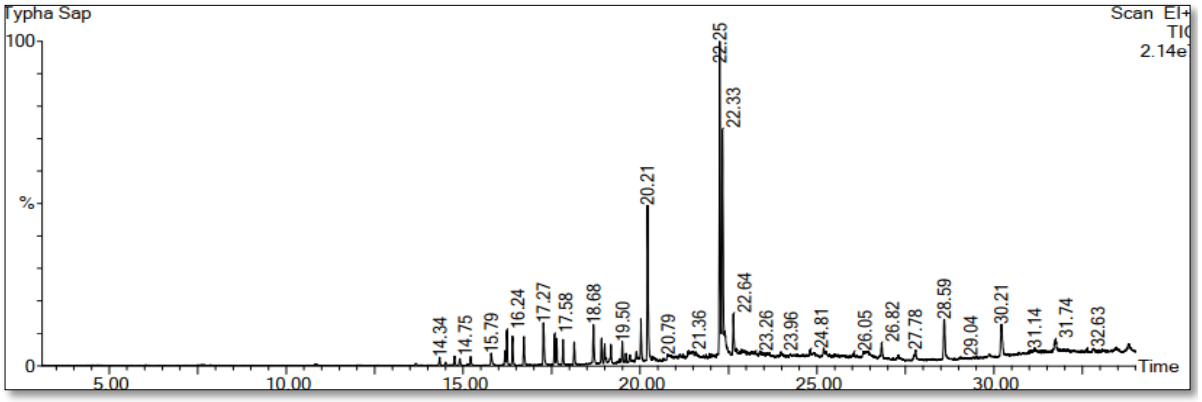


Figure 1: GC/MS total ion chromatogram of TDHF- SAP.

Table1: GC/MS analysis of compounds detected in TDHF-SAP

Compound No.	Rt (min)	Name	$[m]^+$ *m/z*	Molecular formula	Peak area %	**Prob.**
1.	16.239	Benzeneacetic acid, 2-propenyl ester	176	$C_{11}H_{12}O$	1.138	9.9
2.	19.600	6-Octen-1-ol, 3,7-dimethyl-, propanoate	212	$C_{13}H_{24}O$	0.406	15.5
3.	19.715	Oxalic acid, allyl pentadecyl ester	340	$C_{21}H_{38}O_4$	0.433	9.1
4.	20.210	Tridecanoic acid methyl ester	228	$C_{14}H_{28}O_2$	6.596	30.8
5.	20.786	Oxalic acid, cyclobutyl octadecyl ester	396	$C_{24}H_{44}O_4$	0.630	10.4
6.	21.026	Oxalic acid, allyl octadecyl ester	382	$C_{23}H_{42}O_4$	0.422	19.0
7.	21.116	Octadecanoic acid, 2-oxo-, methyl ester	312	$C_{19}H_{36}O$	0.538	13.8
8.	21.211	Nitric acid, nonyl ester	189	$C_9H_{19}NO$	0.519	10.4
9.	21.441	Methyl 2-hydroxydodecanoate	230	$C_{13}H_{26}O_3$	0.483	8.1
10.	21.491	Cyclohexanecarboxylic acid, 4-heptyl-, 4-Fluorophenyl ester	320	$C_{20}H_{29}FO_2$	0.610	46.1
11.	21.571	Hexadecanoic acid, 2-oxo-, methyl ester	144	$C_7H_{12}O_3$	0.977	6.1
12.	22.251	9,12-Octadecadienoic acid, methyl ester, (*E*,*E*)	294	$C_{19}H_{34}O$	11.623	52.3
13.	22.331	9-Octadecenoic acid (*Z*)-, methyl ester	296	$C_{19}H_{36}O_2$	9.502	40.8
14.	22.401	Pentanoic acid, 10-undecyl ester	256	$C_{16}H_{32}O_2$	0.418	9.8
15.	22.641	Hexadecanoic acid, 15-methyl-, methyl ester	284	$C_{18}H_{36}O_2$	1.961	26.2
16.	24.812	Oxalic acid, allyl hexadecyl ester	354	$C_{21}H_{38}O_4$	0.458	14.5
17.	25.187	Hexadecanoic acid, 2-hydroxy-, methyl ester	286	$C_{17}H_{34}O_3$	0.513	39.7
18.	26.318	Malonic acid, benzyl-, di-(-)-menthyl ester	354	$C_{22}H_{26}O_4$	0.489	56.4
19.	27.298	Oxalic acid, allyl tridecyl ester	312	$C_{18}H_{32}O_4$	0.403	17.5
20.	27.778	Didodecyl phthalate	502	$C_{32}H_{54}O_4$	1.026	20.4
21.	28.599	Ethanedioic acid, bis(trimethylsilyl) ester	234	$C_8H_{18}O_4Si_2$	2.815	17.2
22.	29.874	Dichloroacetic acid, 2,2-dimethylpropyl ester	199	$C_7H_{12}Cl_2O_2$	0.404	11.5
23.	31.735	Methanol, [4-(1,1-dimethylethyl)phenoxy]-, acetate	222	$C_{13}H_{18}O_3$	1.736	29.9

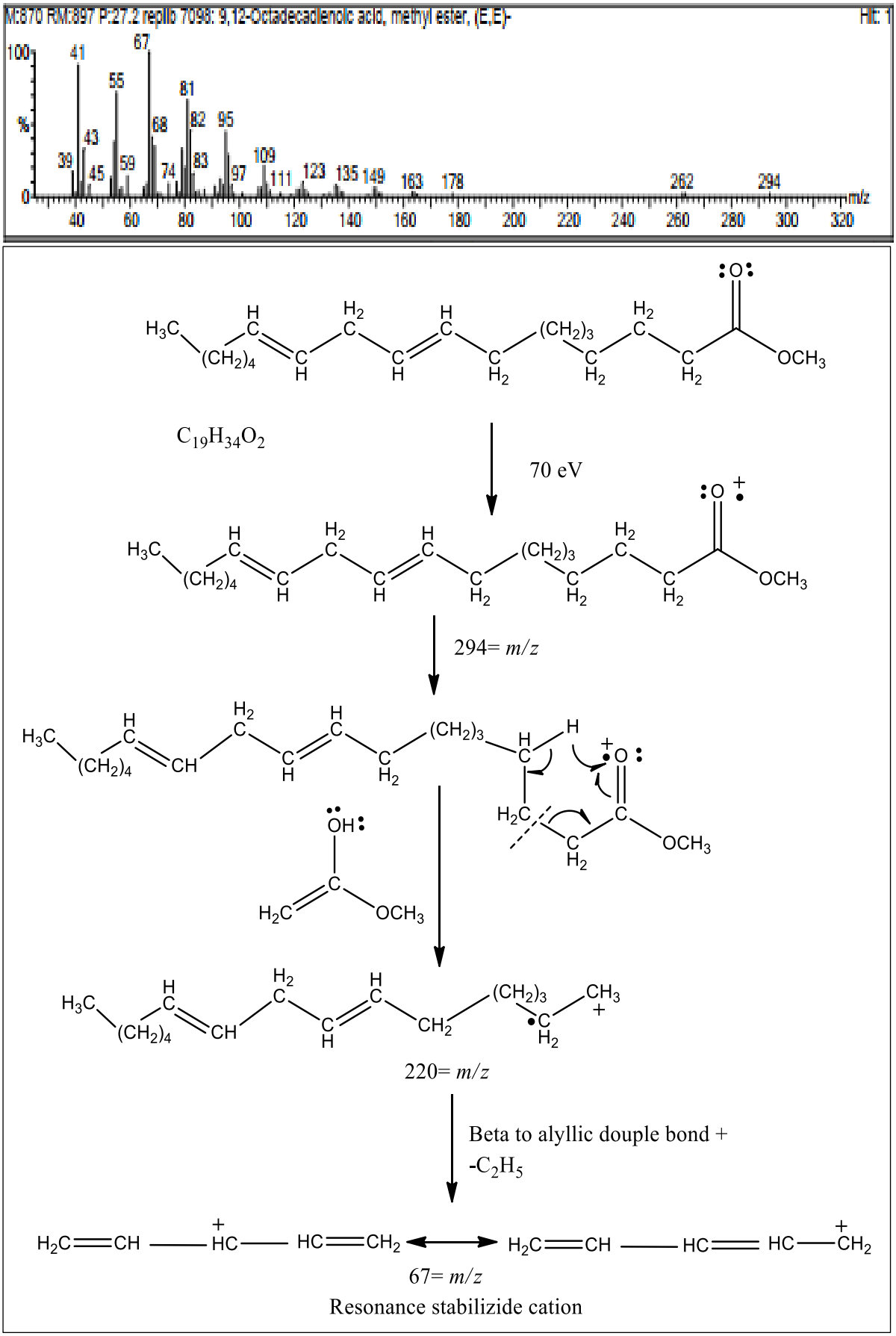


Figure 2: Structure and mass fragmentation patterns of 9, 12-Octadecadienoic acid, methyl ester, (*E*,*E*)

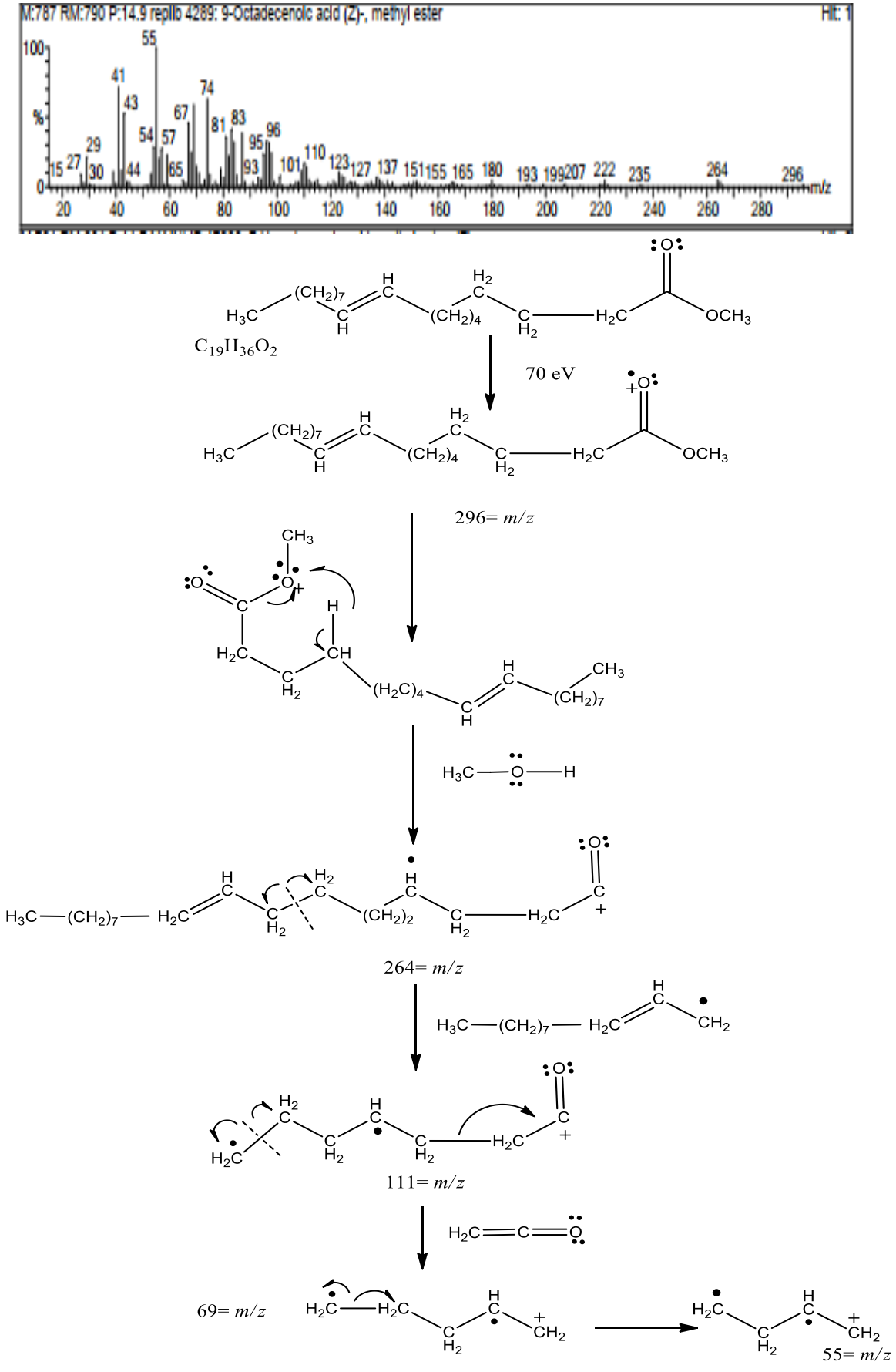
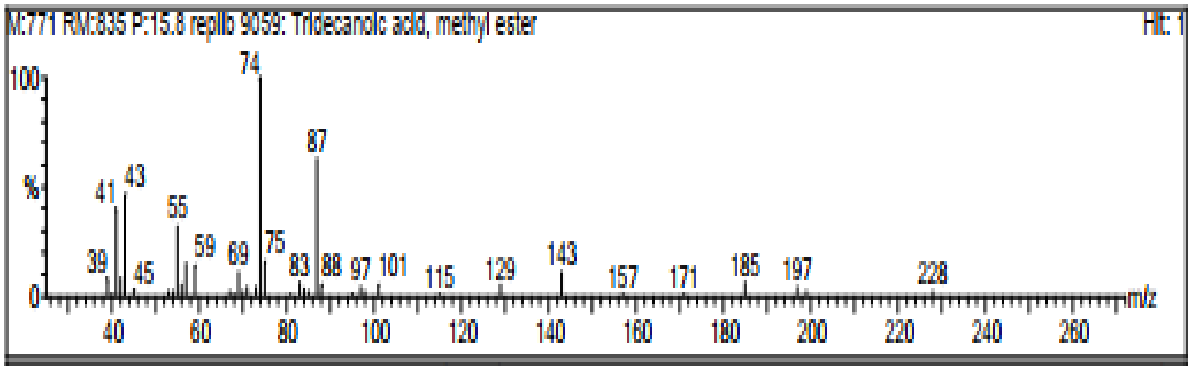


Figure 3: Structure and mass fragmentation patterns of 9- Octadecadienoic acid (*Z*), methyl ester



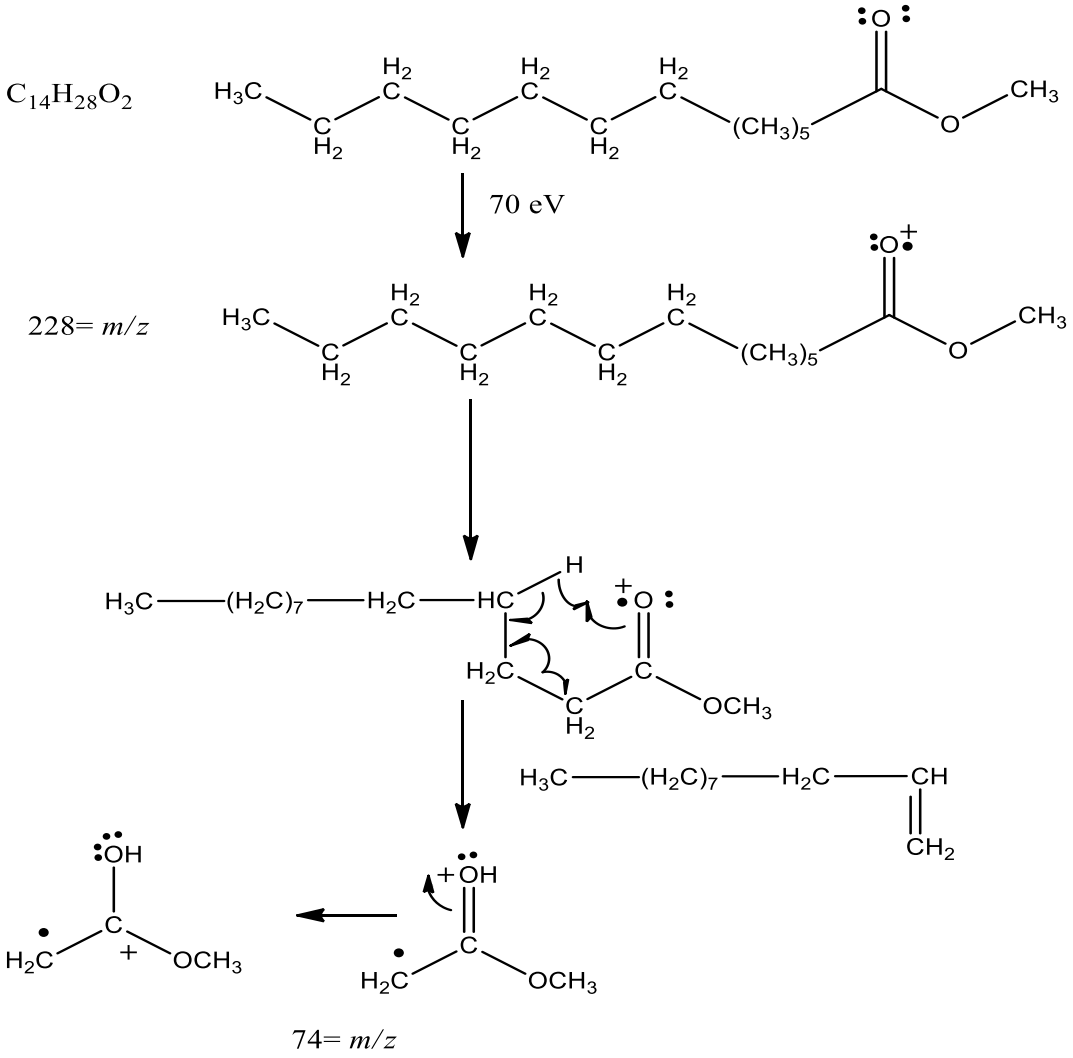


Figure 4: Structure and mass fragmentation patterns of Tridecanoic acid, methyl ester.

3.4. Antimicrobial activity

The antimicrobial efficacy of TDME and its different fractions was evaluated as mentioned before[17]. As illustrated in **Figure (5)** and **Table (2),** TDME had good antibacterial activity which can be compared to standard drug (ampicillin). TDME had antibacterial activity against *P. aeruginosa* and *S. aureus* with MIC values 4 µg/mL and 2 µg/mL, respectively compared to ampicillin with MIC values 2 µg/mL and 1 µg/mL. TDME also had antibacterial activity against *B. subtilis* with MIC value 4 µg/mL that was equal to the MIC value of ampicillin.

The antimicrobial results of *T. domingensis* Pers. were near to what was found by Al-Kalifawi et al. (2017) [4], who demonstrated antimicrobial activities of different extracts of *T. domingensis* Pers. against several Gram-positive and Gram-negative bacteria. The results of (MIC) of *T. domingensis* Pers. leaves extracts on different microbes isolates showed methanol extract of *T. domingensis* Pers. exhibited the highest antimicrobial activity against bacteria and yeasts followed by aqueous extract while the chloroform extract was less effective.

This is the first time to evaluate the antimicrobial activity of TDME different fractions, as illustrated in **Figure (5, 6)** and **Table (2).** TDEF had the most powerful antibacterial effect among the tested fractions. TDEF had inhibitory effect against B. subtilis with MIC value (2 µg/mL) that was half to that of the control (4 µg/mL). Also, this fraction had the inhibitory effect against *P. aeruginosa* and *S. aureus* with MIC values equal to that of the control.

TDME and its different fractions had modest inhibitory effect against the tested fungi **(Figure 6).**

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Table 2. MIC values (µg/mL) ± SD for TDME and its different fractions against several bacterial and fungal strains. Using ampicillin and clotrimazole as positive control. Results are the mean of three repetitions. (n = 3).

Compounds	MIC values (µg/mL)					
	Mean ± SD					
	E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*C. Albicans*	*A .flavus*
TDME	8 ± 0.28	4 ± 0.23	2 ± 0.12	4 ± 0.15	32 ± 0.42	16 ± 0.33
TDHF	32 ± 0.25	16 ± 0.31	8 ± 0.11	32 ± 0.4	32 ± 0.53	64 ± 0.51
TDMF	4 ± 0.26	2 ± 0.13	2 ± 0.14	4 ± 0.23	4 ± 0.27	8 ± 0.25
TDEF	2 ± 0.16	2 ± 0.17	1 ± 0.14	2 ± 0.2	4 ± 0.19	2 ± 0.11
TDBF	8 ± 0.24	4 ± 0.27	4 ± 0.1	8 ± 0.31	16 ± 0.27	16 ± 0.29
Ampicillin	0.5 ± 0.01	2 ± 0.03	1 ± 0.05	4 ± 0.04	----	----
Clotrimazole	----	----	----	----	2 ± 0.07	1 ± 0.08

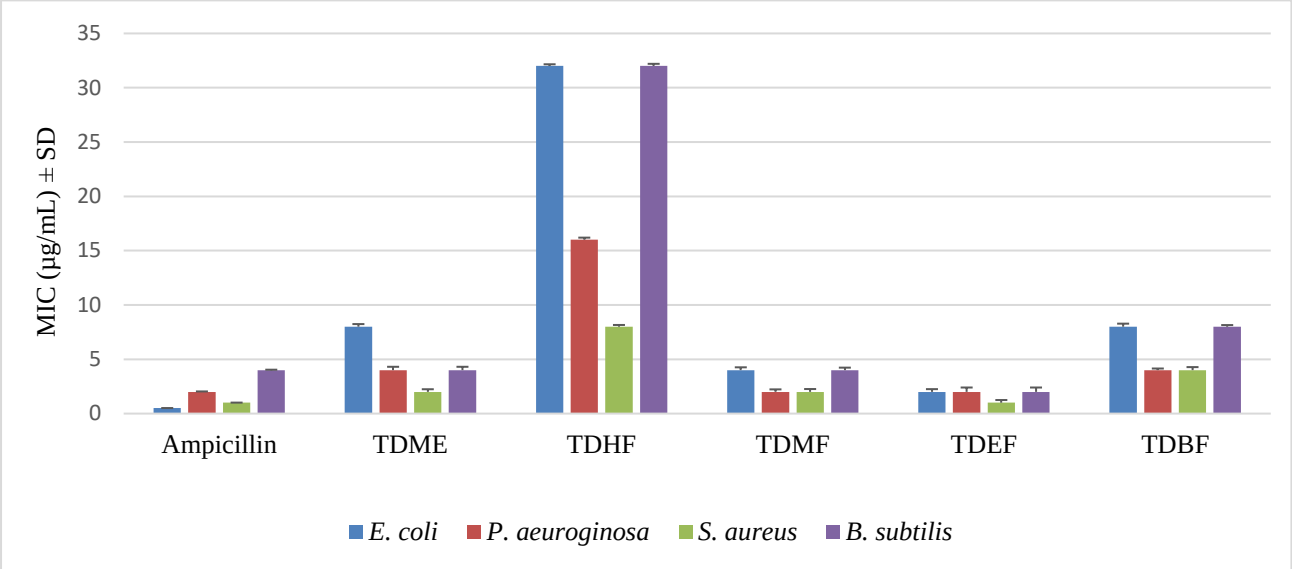

Figure 5. MIC values (µg/mL) ± SD for TDME and its different fractions several bacterial strains and ampicillin was a positive control.

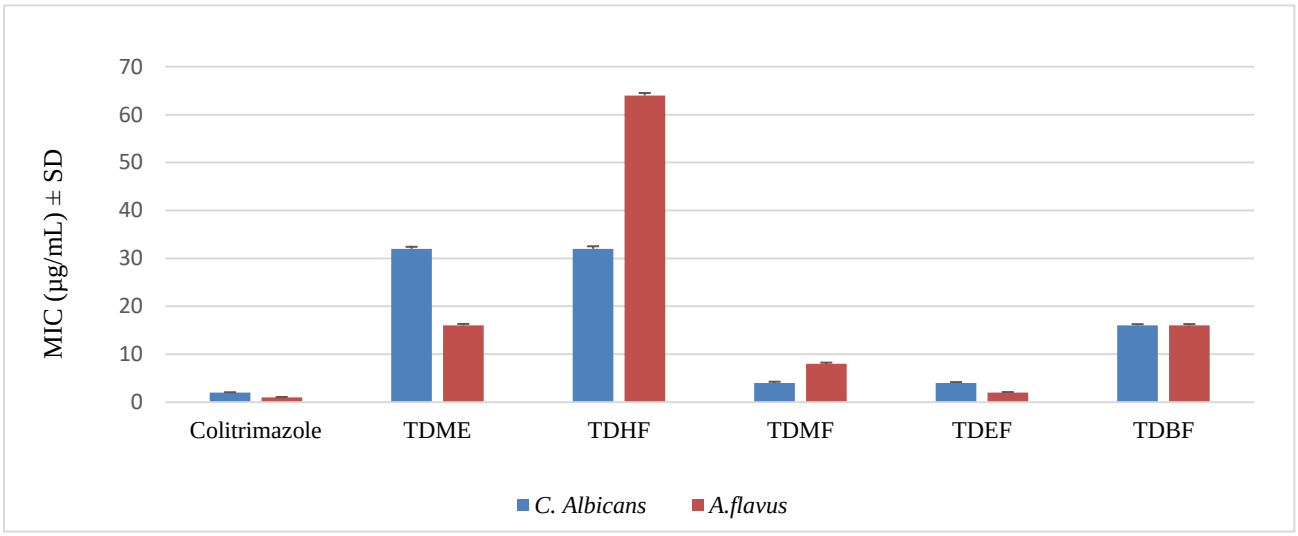

Figure 6. MIC (µg/mL) values ± SD for TDME and its different fractions against *C. albicans*, *A. flavus* and clotrimazole was a positive control.

3.5. TDEF total phenolic and flavonoid contents.

Phenolic substances originating from plants, such as tannins, flavonoids, phenolic acids, and stilbenes, have demonstrated the capacity to prevent a variety of bacteria from growing and acting[23–25]. Flavonoids and phenolic compounds exhibit significant antibacterial effects against several strains of bacteria, particularly *B. subtilis* which was the most affected strain in our study. Their mechanisms of action and efficacy have been the subject of various studies, highlighting their potential as natural antimicrobial agents [26].

The study found that TDME had a total phenolic content of 158.39 ± 1.867 µg gallic acid / mg extract and a total flavonoid content of 40.58 ± 0.4098 µg rutin/ mg. extract. So, we assumed that the antimicrobial activity exhibited by TDME can be linked to elevated levels of flavonoid and phenolic compounds.

As TDEF had the most powerful antibacterial activity among the tested fractions, this encouraged us to determine its total phenolic and flavonoid contents to know the effect of its phenolic and flavonoid contents on its antibacterial activity.

Considering the findings, the total phenolic content of TDEF is 130.138 ± 1.693 expressed as µg gallic acid /mg extract. The total flavonoid content of TDEF is 34.467 ± 1.114 expressed as µg rutin / mg extract.

As illustrated in **Figure (7, 8)** The TDEF possesses a significant portion of total phenolic and flavonoid contents found in the TDME, demonstrating its importance in the plant's overall phytochemical profile. This indicates that TDEF retains a substantial amount of the phenolic compounds, which are known for their antimicrobial properties, suggesting that it could be the reason for the antibacterial activity of this fraction.

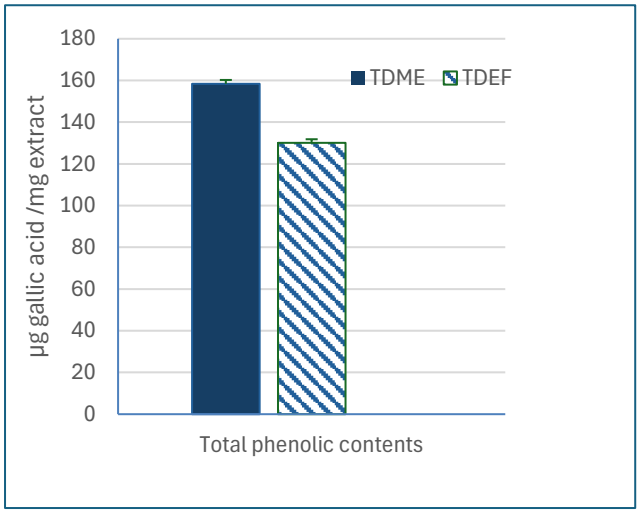

Figure 7: Comparison between total phenolic content of TDME and TDEF.

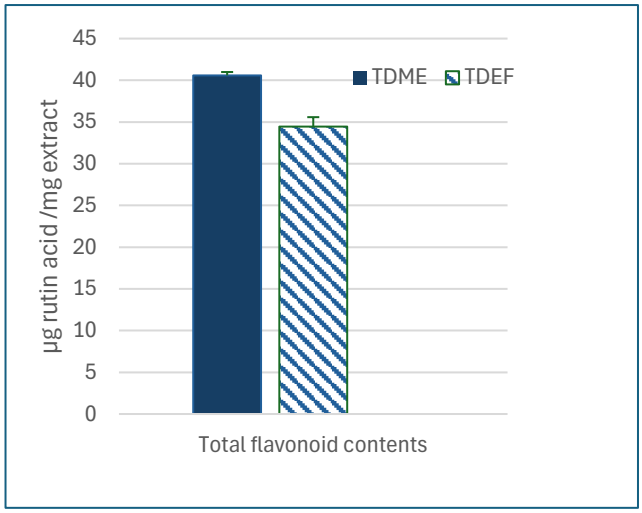

Figure 8: Comparison between total flavonoid content of TDME and TDEF.

CONCLUSION

The research on *Typha domingensis* Pers. underscores its potential as a source of natural antimicrobial agents, revealing significant antibacterial properties linked to its high phenolic and flavonoid contents. GC-MS analysis of TDHF-SAP indicated the existence of twenty-three compounds, with notable compounds exhibiting various biological activities. The total methanol extract (TDME) demonstrated comparable efficacy to standard antibiotics against several bacterial strains, particularly Gram-positive bacteria like *B. subtilis*. The ethyl acetate fraction (TDEF) showed the most potent antibacterial effects, suggesting that the plant could serve as a valuable alternative in combating multidrug-resistant infections. Further studies are needed to elucidate the underlying antimicrobial mechanisms and explore the potential therapeutic applications of the isolated pure compounds from *T. domingensis* Pers. fractions, particularly TDEF.

CONFLICT OF INTEREST

No declared conflict of interest is present between the writers.

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