Pak. J. Chem. 15(1-2): 1-5, 2025 Research Article
ISSN (Print): 2220-2625
ISSN (Online): 2222-307X DOI: 10.15228/2025.v15.i1-2.p01

GC-MS Profiling and Tentative Identification of Bioactive Compounds in Root extract of *Typha elephantina*

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Abstract
The identification of secondary metabolites in plants is a crucial aspect of understanding their biochemical profiles and their ecological, pharmacological, and nutritional roles. *Typha elephantina* is a medicinally potent plant. The study aims to identify a number of bioactive compounds from *Typha elephantina* root extract by using GC-MS analysis. The 10 compounds 1-amino-2-(3-phenoxy benzyl) anthracene-9,10-dione, 4-(ter-butyl)benzyl 3-(2,5-di-tertbutyl phenoxy) benzoate, 4,6-cholestadiene-3one, imino azirin, Desethyl amiodarone benzophenone, Disulfide di hexadecyl, 7-(tert-butyl)-9-(2-methylheptan-2-yl)-1-oxaspiro[4,5]deca-6,9-diene-2,8-dione, Methyl 3-(3,5-di-tert-butyl-4-hydroxy phenyl) propanoate, Pentadecane, 1-(4-isopropyl phenethyl)-7,7 dimethyl bicyclo [2.2.1] heptane-2-ol, 16-oxo-16-phenylhexadec-12-en-1-yl 2-methoxy acetate were identified from TER1(Typha *elephantina* roots extract), immense importance to humans, particularly in the fields of medicine, agriculture, food, and cosmetics. Many of these compounds possess potent pharmacological properties, such as antimicrobial, anticancer, anti-inflammatory, and antioxidant activities, making them valuable for drug development and natural health products. Additionally, they play a vital role in cultivating crop resilience against biotic and abiotic stresses, which has implications for sustainability.
Keywords: Bioactive compounds, *Typha elephantina* roots, fragmentation, structure elucidation, GC-MS analysis.

1.0. INTRODUCTION

Plants are an essential source for discovering new products of medical importance for drug development, and plant secondary metabolites are a unique source of pharmaceuticals, food additives, flavoring, and other industrial value. The viable position of these secondary metabolites has sparked great interest in their production and the exploration of opportunities to improve their production[1].

Medicinal plants play a variety of significant roles in the food and pharmaceutical industries. Plant-derived phytochemical substances have been used to create commercial medicine and have long served as a source for the development of novel pharmaceuticals. It is well established that bioactive substances derived from plants can benefit human health, particularly in the treatment of oxidative stress-related illnesses. The most excellent class of polyphenolic chemicals found in medicinal plants in high concentrations are flavonoids[2]. These substances have been shown to have therapeutic benefits on their own and play a significant role in the plants' well-known pharmacological qualities. The medicinal properties of several polyphenols have been investigated, and the majority of the substances have been turned into brand-name medications for a range of illnesses[3].

The capacity to neutralize free radicals is essential for a bioactive compound to demonstrate pharmacological effects. Free radicals are generated as a result of the body's regular physiological processes; however, they can be regulated under typical circumstances thanks to the antioxidant defense system, which includes reduced glutathione and various antioxidant enzymes[4].

Excessive production of free radicals occurs under adverse conditions, including the consumption of toxic substances or exposure to radiation. This situation disrupts the body's antioxidant defense mechanisms, resulting in oxidative stress. The presence of numerous free radicals at the cellular level contributes to an elevated formation of malondialdehyde (MDA), which is a byproduct of lipid peroxidation occurring at the cell membrane. This process initiates a series of events that may result in tissue damage and potentially lead to organ failure. Lipid peroxidation at the cellular level signifies the activation of inflammatory responses and apoptotic processes[5]. Oxidative stress and inflammation continue to be significant pathophysiological contributors to the onset of various diseases, such as cancer, liver injury, diabetes, neurological disorders, and cardiovascular issues[6]. Bioactive compounds derived from plants represent some of the most promising candidates for the development of drugs aimed at combating diseases associated with oxidative stress due to their antioxidant properties. Research has demonstrated that the external administration of antioxidants can enhance the body's intrinsic antioxidant defense system, as these compounds are capable of traversing the gastrointestinal barrier without being modified by cytochrome P-450 enzymes, which would otherwise lead to their elimination from the body. Furthermore, bioactive compounds identified as xenobiotics in the liver may influence the activity of cytochrome P-450 by evading biotransformation, thereby remaining in their original chemical form and effectively reaching the intended target site[7].

Crude extracts derived from medicinal plants often demonstrate synergistic effects related to antioxidant activities while exhibiting minimal or no toxicity, particularly in preclinical evaluations. Bioactive compounds, notably flavonoids, are frequently developed into therapeutic medications; however, the bioavailability of these individual compounds in vivo studies continues to pose a challenge. Consequently, the use of crude extracts or combinations of bioactive compounds may be considered an effective strategy to address the issue of bioavailability[8].

The aquatic plant *Typha elephantina* (Typhaceae) has exceptional therapeutic properties. Traditionally, almost all parts of *Typha elephantina* have been used to treat various ailments such as boils, burns, wounds, scab problems, bleeding disorders, bacterial infections, leprosy, cystitis, enlarged spleen, strangulation, and many more. Furthermore, the plant is believed to possess many medicinal properties, including membrane stabilizing, thrombolytic, anthelmintic, antioxidant, anxiolytic, wound healing, anti-inflammatory, cytotoxic, and analgesic effects, all of which are supported by pharmacological methods[9].

The aim of the study was to identify chemical constituents from *Typha elephantina* root in n-hexane extract by using GC-MS.

2.0. MATERIAL AND METHOD

Fresh plant *Typha elephantina* (Kondr, Lukhy) was collected from Zhob (daeragi pull, Bhatyie) Balochistan, Pakistan. Identification of the collected plants was conducted through the Plant Sciences Department at Quaid-i-Azam University, Islamabad.

2.1. Extraction

The 20g of *Typha elephantina* root were soaked in 100 ml of n-hexane, and then the filtrate was concentrated by rotary evaporator. The clear and un-turbid samples were prepared for GC-MS Analysis.

2.2. Thin Layer Chromatography

The Thin-layer chromatography was performed by cutting the TLC properly, and samples were poured into it. Then, the TLC was placed inside the mobile phase tank. The mobile phase used was Toluene: Acetone (12:8), and the TLC bands were observed under UV.

2.3. GC-MS Analysis

Gas Chromatography Spectrometry is a powerful analytical technique used for identifying and quantifying chemical compounds in a sample, especially in complex mixtures. It combines two separate techniques, Gas Chromatography (GC) and Mass Spectrometry (MS) to provide detailed information about the composition of the sample.

Table 1. The parameters of GC-MS Instrument

Parameters of GC-MS	
GC-System	Agilent 6890 N
Capillary column	Agilent JW SCIENTIFIC DB-5MS column 30m length, 0.25 mm inner diameter, and 0.5µm film thickness.
Stationary phase	(5% phenyl)-methyl polysiloxane
Carrier gas	Helium (1.5 mL/min)
Column temperature for 17 min	120 C to 280 C
Ramping temperature	10 C per mint
Volume of injected sample	5 µL
Mode of injection of sample	Split mode
Injection pressure	60-85 *psi*
Ionization source	Electron impact (EI)
Electron ionization energy	70 eV
MS analyzer	5973 N Single Quadruple
MS detector	Electron multiplier
Mass range	0-1000 amu

3.0. RESULTS AND DISCUSSION

A small amount of extract was prepared and placed for thin layer chromatography where 4 bands appeared: The purple band with R_f =0.91, a blue band with R_f= 0.8, a light blue band with R_f =0.78, and a yellow band with R_f = 0.65. GC-MS is used to identify the compounds. The Total Ion Chromatogram (TIC) profile showed the elution of 10 compounds with their specific retention time. The 10 compounds were identified by using the GC-MS library and the NIST website as a reference source. The interpretation of (10) compounds is described in Table (2).

Table 2. The identified compounds from **TER-1** *Typha elephantina* root extract.

S.No	Names of compounds	Retention time	Molecular ion peak	Fragment ions
TER1-1	1-amino-2-(3-phenoxy benzyl) anthracene-9,10-dione	4.90	405	372, 343, 305,281,263, 230, 211, 200, 183, 170, 153, 141, 128, 111, 94, 76, 66, 51
TER1-2	4-(terr-butyl)benzyl 3-(2,5-di-tertbutyl phenoxy) benzoate	5.87	472	461, 426, 411, 397, 321, 283, 257, 241, 206, 191,172, 149, 134, 116, 101, 81, 69, 57
TER1-3	4,6-cholestadiene-3one, imino azirin	6.7	435	385, 363, 352, 309, 289, 278, 268, 252, 230, 187, 158, 147, 126, 113, 98, 85, 71, 57
TER1-4	4-benzoyl phenyl desethyl amiodarone	7.4	797	751, 698, 666, 578, 513, 461, 421, 366, 342, 316, 294, 268, 234, 182, 160, 139, 105, 77, 51
TER1-5	1,2-Dihexadecyldisulfane	8.8	514	466, 438, 407, 386, 351, 330, 282, 254, 193, 160, 140, 111, 85, 57
TER1-6	7-(tert-butyl)-9-(2-methyl heptane-2-yl)-1-oxaspiro[4,5]deca-6,9-diene-2,8-dione	10.1	332	301, 273, 232, 219, 205, 192, 177, 162, 149, 135, 109, 96, 79, 55
TER1-7	Methyl 3-(3,5-di-tert-butyl-4-hydroxy phenyl) propanoate	10.2	292	292, 277, 234, 219, 203, 190, 175, 161, 147, 128, 115, 101, 88, 74, 57
TER1-8	Pentadecane	10.8	212	198, 183, 156, 112, 99, 85, 71, 57
TER1-9	1-(4-isopropyl phenethyl)-7,7 dimethylbicyclo[2.2.1]heptan-2-ol	11. 7	286	242, 211, 196, 168, 153, 138, 121, 108, 95, 81, 67, 55
TER1-10	16-oxo-16-phenylhexadec-12-en-1-yl 2-methoxy acetate	12.6	402	390, 343, 296, 279, 259, 209, 183, 146, 127, 111, 98, 85, 71, 57

The molecular ion peak appeared at *m/z* 405 yielded fragment ions *m/z* 372, *m/z* 343,*m/z* 305, *m/z* 281 and *m/z* 263 with the loss of 33 amu [M-H-NH_2OH], 62 amu [M-H-C_5H_2], 100 amu [M-H-$C_6H_5OH+3H_2$], 124 amu [M-H-$C_8H_{12}O$] and142 amu [M-H-C_9H_2O2]. The daughter ion peaks *m/z* 230, *m/z* 211, *m/z* 200, *m/z* 183 and *m/z* 170 were generated by the loss of 175 amu [M-H-$C_{11}H_{11}O_2$], 194 amu[M-H-$C_{12}H_4NO_2$], 205 amu [M-H-$C_{15}H_9O$], 222 amu [M-H-$C_{14}H_8NO_2$] and 235 amu [M-H-$C_{15}H_9NO_2$]. The *m/z* 170 is the characteristic of diphenyl ether confirmed from GC-MS library matches and NIST mass spectrum, so further fragmentation was done from *m/z* 170. *m/z* 153, *m/z* 141 and *m/z* 128 appeared with the loss of 17 amu [OH], 29 amu [CHO] and 42 amu [C_3H_6]. The *m/z* 111 was obtained from 128 with the loss of 17 amu [OH]; the last four fragment ions were obtained at *m/z* 94, *m/z* 76, *m/z* 66, and *m/z* 51 upon the loss of 76 amu [C_6H_4], 94 amu [C_6H_5OH], 104 amu [C_7H_4O] and 119 amu. From the above fragmentation, the compound (1) was considered as 1-amino-2-(3-phenoxy benzyl) anthracen 9,10 dione.

The molecular ion displayed at *m/z* 472. The fragment ions were produced at *m/z* 461, *m/z* 426, *m/z* 411, *m/z* 397, *m/z* 321, *m/z* 283 and *m/z* 257, *m/z* 241 and *m/z* 206 upon the loss of 11amu [M-11H], 46 amu [CO_2+H_2], 61 amu [M-C_4H_{13}], 75 amu [M-C_5H_{15}], 151 amu [M-$C_{11}H_{15}$], 189 amu [M-$C_{12}H_{13}O_2$], 215 amu [M-$C_{14}H_{15}O_2$], 231 amu [M-$C_{15}H_{19}O_2$] and 266 amu [M-$C_{18}H_{18}O_2$]. The *m/z* 206 is the characteristic of 2,5-di-tert-butyl phenol confirmed from the GC-MS library and NIST mass spectrum. Therefore, further fragmentation was done from *m/z* 206. The *m/z* 191, *m/z* 172, *m/z* 149, *m/z* 134, and *m/z* 116 were obtained by the loss of 15 amu [CH_3], 34 amu [$2CH_3+2H_2$], 57 amu [C_4H_9], 72 amu [C_5H_{12}] and 90

amu [C_7H_6]. The *m/z* 101 was brought from *m/z* 116 with the loss of 15 amu [CH_3]. The last 3 fragments appeared at *m/z* 81, *m/z* 69 and *m/z* 57 by the loss of 125 amu [$C_{18}H_{13}O$], 137 amu [$C_9H_{13}O$] and 149 amu [$C_{10}H_{13}O$]. The compound (2) was assigned as 4-(ter-butyl)benzyl 3-(2,5-di-tertbutyl phenoxy) benzoate.

The compound (3) showed a molecular ion peak at *m/z* 435. The fragment ion generated at *m/z* 385 and *m/z* 363 with the loss of 50 amu[M-C_4H_2] and 72 amu [M-$C_3H_8N_2$]. The further fragmentation takes place from *m/z* 363, according to the library, which matches it near the original compound. The fragment ions *m/z* 352, *m/z* 309, *m/z* 289, *m/z* 278, *m/z* 268, *m/z* 252, *m/z* 230, *m/z* 187, *m/z* 158 and *m/z* 147 with the loss of 11 amu [11H], 54 amu [C_4H_6], 74 amu [$C_4H_{10}O$], 85 amu [C_5H_9O], 95 amu [C_6H_7O], 111 amu [C_8H_{15}], 133 amu [$C_{10}H_{13}$], 176 amu [$C_{12}H_{16}O$], 205 amu [$C_{14}H_{21}O$] and 216 amu [$C_{16}H_{24}$]. The *m/z* 126, *m/z* 113, *m/z* 98, *m/z* 85, *m/z* 71 and *m/z* 57 were produced by the loss of 237 amu [$C_{17}H_{17}O$], 250 amu [$C_{18}H_{18}O$], 265 [$C_{20}H_{25}$], 278 amu [$C_{21}H_{26}$], 292 amu [$C_{22}H_{28}$] and 306 amu [$C_{23}H_{30}$]. Compound (3) was identified as 4,6-cholestadiene-3one, imino azirin.

The molecular ion appeared at *m/z* 797 yielded fragment ions *m/z* 751, *m/z* 698, *m/z* 666, *m/z* 578, *m/z* 513, *m/z* 461 and *m/z* 421 with the loss of 46 amu [M-C_2H_8N], 99 amu [M-C_5H_9NO], 131 amu [M-I+$3H_2$], 219 amu [M-C_6H_4OI], 284 amu [M-C_2H_6+I_2], 336 amu [M-$C_6H_{10}I_2$] and 376 amu [M-$C_7H_8ONI_2$]. *m/z* 366, *m/z* 342, *m/z* 294, *m/z* 268, *m/z* 234 and *m/z* 182 appeared upon the loss of 431 amu [M-$C_{10}H_{11}I_2NO_2$], 455 amu [M-$C_{12}H_{11}I_2NO_2$], 503 amu [M-$C_{16}H_{11}I_2NO_2$], 529 amu [M-$C_{18}H_{13}I_2NO_2$], 563 amu [M-$C_{19}H_{19}I_2NO_3$] and 615 amu [$C_{23}H_{23}I_2NO_3$]. *m/z* 316 was obtained by the loss of 26 amu [C_2H_2] from *m/z* 342. Further fragmentation was done from *m/z* 182, which was the characteristic of benzophenone, as confirmed by the GC-MS library and NIST mass spectrum. The *m/z* 160, *m/z* 139, *m/z* 105, *m/z* 77 and *m/z* 51 were generated with the loss of 22 amu [H_2O+$2H_2$], 43 amu [C_2H_3O], 77 amu [C_6H_5], 105 amu [C_7H_5O] and 131 amu [C_9H_7O]. Compound (4) was considered as 4-benzoylphenyl desethyl amiodarone.

The molecular ion for compound (5) displayed at *m/z* 514. The *m/z* 466 appeared by the loss of 48 amu [C_3H_8+$2H_2$]. The *m/z* 438 was obtained by the loss of 28 amu [C_2H_4] from *m/z* 466. The *m/z* 407, *m/z* 386, *m/z* 351, *m/z* 330, *m/z* 282, *m/z* 254 and *m/z* 193 were produced with the loss of 107 amu [M-C_7H_{15}+$4H_2$], 128 amu [M-C_9H_{20}], 163 amu [M-$C_{11}H_{23}$+$4H_2$], 184 amu [M-$C_{13}H_{28}$], 232 amu [M-$C_{16}H_{34}$+$3H_2$], 260 amu [$C_{16}H_{34}S$+H_2] and 321 amu [$C_{18}H_{37}S_2$+$2H_2$]. *m/z* 160, *m/z* 140, *m/z* 111, *m/z* 85 and *m/z* 57 were produced with the loss of 354 amu [M-$C_{20}H_{42}S_2$+$4H_2$], 374 amu [M-$C_{22}H_{46}S_2$], 403 amu [M-$C_{24}H_{49}S_2$+H_2], 429 amu [M-$C_{26}H_{53}S_2$] and 457 amu [M-$C_{28}H_{57}S_2$]. From library matches and the NIST mass spectrum, compound (5) was considered as 1,2-Dihexadecyldisulfane.

The molecular ion of compound (6) appeared at *m/z* 332 and yielded fragment ions *m/z* 301 and *m/z* 273 with the loss of 31 amu [M-C_2H_5+H_2] and 59 amu [M-C_4H_{11}]. Further fragmentation was done from *m/z* 273 because, according to library matches and the NIST mass spectrum, it was nearer to the original compound. The *m/z* 232, *m/z* 219, *m/z* 205, *m/z* 192, *m/z* 177 and *m/z* 162 were generated with the loss of 41 amu [C_3H_5], 54 amu [C_4H_6], 68 amu [C_5H_8], 81 amu [C_6H_9], 96 amu [C_6H_8O] and 111 amu [$C_7H_{11}O$]. *m/z* 135 and *m/z* 149 were obtained from *m/z* 177 with the loss of 42 amu [C_3H_6] and 28 amu [CO]. The *m/z* 109, *m/z* 96, *m/z* 79, and *m/z* 55 were produced by the loss of 164 amu[$C_{10}H_{12}O_2$], 177 amu [$C_{12}H_{17}O$], 194 amu [$C_{11}H_{14}O_3$] and 218 amu [$C_{13}H_{14}O_3$]. The compound (6) was considered as 7-(tert-butyl)-9-(2-methylheptan-2-yl)-1-oxaspiro[4,5]deca-6,9-diene-2,8-dione.

The molecular ion for compound (7) appeared at *m/z* 292. The fragment ions were generated at *m/z* 277, *m/z* 234, *m/z* 219, *m/z* 203, and *m/z* 190 with the loss of 15 amu [M-CH_3], 58 amu [M-$C_2H_2O_2$], 73 amu [M-$C_3H_5O_2$], 89 amu [M-$C_4H_9O_2$] and 102 amu [M-C_5H_{10}]. The *m/z* 175 was obtained from *m/z* 190 with the loss of 15 amu [CH_3]. The *m/z* 161 was due to a loss of 131 amu [$C_7H_9O_2$+$3H_2$]. The 147 was brought from *m/z* 175 with the loss of 28 amu [CO]. The *m/z* 128 appeared with the loss of 33 amu [CH_5O] from *m/z* 161. *m/z* 115, *m/z* 101, *m/z* 88, *m/z* 74 and *m/z* 57 resulted by the loss of 177 amu [M-$C_{12}H_{17}O$], 191 amu [M-$C_{12}H_{15}O_2$], 204 amu [M-$C_{14}H_{20}O$], 218 amu [M-$C_{15}H_{22}O$], 235 amu [M-$C_{16}H_{27}O$]. The compound (7) was identified as Methyl 3-(3,5-di-tert-butyl-4-hydroxy phenyl) propanoate.

The molecular ion peak for compound (8) is displayed at *m/z* 212. The fragment ions *m/z* 198, *m/z* 183, *m/z* 156, *m/z* 112, *m/z* 99, *m/z* 85, *m/z* 71 and *m/z* 57 were generated with the loss of 14 amu [M-CH_2], 29 amu [M-C_2H_5], 56 amu [M-C_4H_6], 100 amu [M-C_7H_{16}], 113 amu [M-C_8H_{17}], 127 amu [M-C_9H_{19}], 141 amu [M-$C_{10}H_{21}$] and 155 amu [M-$C_{11}H_{23}$]. Compound (8) was considered as Pentadecane.

The molecular ion peak for compound (9) is displayed at *m/z* 286. The fragment ion *m/z* 242 *m/z* was obtained by the loss of 44 amu [M-C_3H_8]. The *m/z* 211 appeared with the loss of 31 amu [C_2H_7]. The *m/z* 196, *m/z* 168, and *m/z* 153 resulted in the loss 90 amu [M-C_7H_6], 118 amu [M-C_9H_{10}], 133 amu [M-$C_{10}H_{13}$]. The further fragmentation was done from *m/z* 153 because it was nearer to the original structure. *m/z* 138, *m/z* 121, *m/z* 108, *m/z* 95, *m/z* 81, *m/z* 67 and *m/z* 55 appeared by the loss of 15 amu [CH_3], 32 amu [C_2H_8], 45 amu [C_2H_5O], 58 amu [C_3H_6O], 72 amu [C_4H_8O], 86 amu [C_6H_{14}] and 98 amu [C_7H_{14}]. The compound (9) was considered as 1-(4-isopropyl phenethyl)-7,7 dimethylbicyclo[2.2.1] heptan-2-ol.

The molecular ion for compound (10) was displayed at *m/z* 402. The fragment ions *m/z* 390, *m/z* 343, *m/z* 296, and *m/z* 279 resulted in the loss of 12 amu [M-C], 59 amu [M-C_3H_7O], 106 amu [M-C_7H_6O], 123 amu [M-C_8H_8O+3H]. Further fragmentation was done from *m/z* 279 due to library matches and the NIST mass spectrum, which was nearer to the original compound. The *m/z* 259, *m/z* 209, *m/z* 183, *m/z* 146, *m/z* 127, *m/z* 98, *m/z* 85, *m/z* 71 and *m/z* 57 were generated by the loss 20 amu [CH_2+$3H_2$], 70 amu [$C_3H_2O_2$], 96 amu [C_7H_{12}], 133 amu[$C_{10}H_{13}$], 152 amu [$C_{11}H_{20}$], 181 amu [$C_{10}H_{13}O_3$], 194 amu [$C_{14}H_{26}$], 208 amu [$C_{12}H_{16}O_3$], 222 amu [$C_{13}H_{18}O_3$]. The compound (10) was assigned as 16-oxo-16-phenylhexadec-12-en-1-yl 2-methoxyacetate.

4.0. CONCLUSION

The 10 compounds 1-amino-2-(3-phenoxy benzyl) anthracene-9,10-dione, 4-(terr-butyl)benzyl 3-(2,5-di-tertbutyl phenoxy) benzoate, 4,6-cholestadiene-3one, imino azirin, Desethyl amiodarone benzophenone, Disulfide dihexadecyl, 7-(tert-butyl)-9-(2-methylheptan-2-yl)-1-oxaspiro[4,5]deca-6,9-diene-2,8-dione, Methyl 3-(3,5-di-tert-butyl-4-hydroxy phenyl) propanoate, Pentadecane, 1-(4-isopropyl phenethyl)-7,7 dimethylbicyclo [2.2.1] heptan-2-ol, 16-oxo-16-phenylhexadec-12-en-1-yl 2-methoxyacetate, were identified from n Hexane root extract of TER1-1 using GC-MS analysis which were reported as bioactive in the literature. In medicine, plant-derived secondary metabolites have already contributed to the development of numerous pharmaceuticals, such as anticancer agents, antibiotics, and anti-inflammatory drugs. The ongoing exploration of plant metabolites offers immense promise for addressing contemporary challenges, from antimicrobial resistance to the search for more sustainable and eco-friendly chemical alternatives. However, further research is needed to fully uncover their potential for biotechnology, medicine, and agriculture.

Acknowledgment

The author acknowledges the Department of Chemistry Lab-53B and the Chairperson of the Department of Chemistry, Quaid-e-Azam University, Islamabad, Pakistan, Dr. Hummaira Sidiqqui, for their support.

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Received: November 30th 2024 **Accepted: December 27th 2024**