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Original Article

Characterization and identification of bioactive natural products in the ethanol extracts of *Acacia nilotica*, *Melia azedarach*, and *Euphorbia hirta* from Cholistan desert, Pakistan

Sobia Malik[1,2], Nuzhat Sial[1], Mirza Imran Shahzad[3*], Shazia Anjum[4], Arshad Javid[5], Gildardo Rivera[6]

[1]Department of Zoology, The Islamia University, Bahawalpur, Pakistan
[2]Department of Zoology, Gover nment Sadiq College Women University, Bahawalpur, Pakistan
[3]Department of Biochemistry, The Islamia University, Bahawalpur, Pakistan
[4]Department of Chemistry, The Islamia University, Bahawalpur, Pakistan
[5]Department of Wildlife and Ecology, University of Veterinary and Animal Sciences, Lahore, Pakistan
[6]Laboratorio de Biotecnología Farmacéutica, Centro de Biotecnología Genómica, Instituto Politécnico Nacional, México

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**Corresponding author email:*
mirza.imran@iub.edu.pk

Abstract

Cholistan desert plants form Fabaceae, Meliaceae and Euphorbiaceae families have always been recognized as an alternate source of medicine and used in different pharmacological activities due to the presence of bioactive secondary metabolites. This study was aimed to characterize bioactive contents in ethanol extracts of *Acacia nilotica* (whole branch, bark), *Melia azedarach* (leaves, bark,) and *Euphorbia hirta* (whole plant). Characterization and composition of secondary metabolites were determined by both chromatographic and non-chromatographic techniques. TLC profile showed maximum spots in *Acacia nilotica* and *M. azedarach*. *A. nilotica* whole branch yielded nine spots for *n*-Hex, seven spots for DCM, 4 spots for EtAC while *A. nilotica* bark yielded 4 spots for *n*-Hex, nine spots for DCM and 4 spots for EtAC. *M. azedarach* leaves revealed seven spots for *n*-Hex, six spots for DCM, seven spots for EtAC, while *M. azedarach* bark revealed seven spots for *n*-Hex, five spots for DCM, and also five spots for EtAC. *E. hirta* yielded six spots for *n*-Hex, two spots for DCM and for EtAC two spots detected. FT-IR spectra showed the characteristic prominent peaks. The maximum number of functional groups were observed in *M. azedarach* bark, followed by *A. nilotica* whole branch/bark and *M. azedarach* whole branch. The least number of functional groups were observed in *E. hirta*. HPLC analysis was revealed that 9 compounds were majorly quantified in *A. nilotica* whole branch bark i.e., Gallic Acid, *p*-hydroxy benzoic acid, Gentisic Acid, Protocatechuic Acid, Catechin, Syringic Acid, Chlorogenic Acid, Vanillic acid, and Epi-catechin. twenty-three compounds were predominantly quantified in *M. azedarach* leaves bark i.e., Quercetin, Hydroxy ferulic acid hexoside, Rutin, Vanillic Acid, Ferulic Acid, Ferulic acid hexoside II, Feruloylquinic Acid, Myricetin hexoside, Kaempferol -3- O -rutinoside, Kaempferol -3- O – rhamnoside, Procyanidin dimer B, Toosendanin, Quercetin-7-O- glycoside, Kaempferol, Catechin-7-O- glycoside, Apigenin -7-O- glycoside, Kaempferol -7-O-glycoside, Catechin-5-O-glycoside, Capric acid methyl ester, 8- Hexadecene, Phytadiene, γ-n-Amyl butyrolactone, Apigenin, Luteolin, Kaempferol -3-O-glycoside and from *E. hirta* seven compounds were quantified i.e., Rutin, Gallic Acid, Tannic Acid, Resorcinol, Ellagic Acid, Benzoic Acid, Quercetin. The LCMS scan of *A. nilotica* whole branch demonstrated the presence of twelve active compounds showing 1.612 -11.183 retention time, fifteen compounds confirmed in *A. nilotica* bark with rt 0.700 - 14.202, *M. azedarach* leaves showed only eleven compounds with rt 1.494 -13.031, *M. azedarach* bark showed 14 compounds in with rt 1.659 - 13.039 and *E. hirta* revealed eleven compounds with rt 1.557 - 10.884. The GCMS scan of *A. nilotica* whole branch ten compounds were detected with rt 23.529 - 35.779, *A. nilotica* bark 9 compounds identified with rt 6.180 - 36.157. Only one compound from *M. azedarach* leaves identified with rt 34.116, while 5 compounds found in *M. azedarach* bark with rt 30.740 - 35.379. *E. hirta* revealed twenty-eight compounds with rt 6.411 – 36.933. The experimental data of studies suggest that the presence of active compound introduce the therapeutic use against infectious diseases and also based on assumption that Cholistan desert medicinal plants are rich source(s) that confers various biological activities.

Keywords: Cholistan Desert, medicinal plants, ethanol extraction, liquid-liquid fractionation, TLC, phytochemical analysis.

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Introduction

Medicinal plants are regarded as the most abundant bioresources for pharmaceuticals due to their use in both traditional and modern medicine, pharmaceutical intermediates, nutraceuticals, nutrition supplements, and chemical components of synthetic medications. Since they are a rapid and effective healthcare option, plants have been utilized to control sickness and have steadily increased throughout time as supplemental medicine (Nafiu et al., 2017). World Health Organization (WHO) estimates that 80% of the population still uses traditional remedies to cure a variety of illnesses (Rachuounyo et al., 2016; Tesfauneygn and Gebreegziabher, 2019). Microorganisms, marine life, and plants are the three primary fundamental sources of natural resources. Since ancient times, medicinal plants have been used for their therapeutic benefits as well as to taste and preserve food (Benarba and Pandiella, 2020; Bagherniya et al., 2021). The secondary metabolites' biological activities and *in-vitro* biological activities have a high association. Since they offer a quick and effective alternative to conventional treatment, plants have been employed in illness management and complementary therapy. On our globe, there are 55000 plant species have been used in alternative medicine (Nafiu et al., 2017; Agca et al., 2021).

The Fabaceae plant *Acacia nilotica* is indigenous to Pakistan and may be found in Khyber Pakhtunkhwa, Sindh, Punjab, and Balochistan. The plant has been discovered to have considerable antibacterial, antioxidant, anti-diarrhea, anti-cancer, antimutagenic, anthelmintic, antiplatelet aggregatory, and vasoconstrictor action, among other qualities. (Goronyo et al., 2022). The Meliaceae family includes a broad variety of floral and fruit structures, and despite extensive research over the years, it continues to be a significant source of compounds with therapeutic potential (Huang et al., 2007). Euphorbia has a wide range of biological and genetic characteristics. The anti-arthritis, anti-inflammatory, anti-microbial, anti-oxidant, anti-pasmodic, anti-tumor, and myelopoiesis effects of euphorbia have been documented (Bani et al., 2007; Ernst et al., 2015). Additionally, the plant has reportedly been shown to provide a variety of therapeutic benefits, including worm infestations, antibacterial, antifungal, antiurolithiatic, analgesic, antimalarial, and antiviral characteristics (Singh and Kumar, 2013; Asha et al., 2014). In this study Cholistan desert plants with medicinal relevance were studied which offer a rich source of medicinal compounds and they became an important approach in the natural bioactive compound's discovery.

Material and Methods

Plant Extraction

Plants were collected from Cholistan Desert near Baghdad Campus of the Islamia University of Bahawalpur, Pakistan. Plants were collected from Cholistan Desert near Baghdad Campus of the Islamia University of Bahawalpur, Pakistan. Plant Taxonomist from Department of Life Sciences, Islamia University of Bahawalpur identified the plant material and voucher specimens AN-1645, MA-1647, and EH-1644 were submitted to herbarium. The 15-day shade dried plant material was ground into 500 g powder, which was then extracted with 1-2 L of ethanol for 7 days and filtered (Ahmad et al., 2014). Each filtrate was concentrated using a rotary evaporator at 30 ºC to 35 ºC (Joel and Bhimba, 2010). The crude extract was mixed with distilled water and fractioned by liquid-liquid partitioning using *n*-hexane, dichloromethane, and ethyl acetate as solvents in that order to separate the chemicals in the crude extract with increasing polarity. The fractions were then dried in the dark,

weighed, and kept at 4°C for phytochemical (Naseer et al., 2014).

Thin Layer Chromatography

Thin Layer Chromatography (silica gel G 60 F254) plates with a layer thickness of 0.2 mm were used to determine the number of spots and Rf values in n-hexane, dichloromethane, and ethyl acetate fractions of crude ethanol extracts. To get the optimum results, various solvent solutions were explored. TLC plates were examined in daylight first, then in a UV chamber, and Rf values were computed. Different solvent systems were discovered to be efficient in obtaining the greatest number of spots for varied extracts (Abdul et al., 2017; Gautam et al., 2022).

High Performance Liquid Chromatography

HPLC quantification of different bioactive plant compounds were performed using (Agilent USA-Agilent 1260 Infinity) instrument equipped with Diode Array Detector (DAD G131D5) was used. HPLC system operated at 35 °C and equipped Agilent with C18 column (100 mm x 4.6 mm). 20 µL injection volume with standard injection mode, 1 mL min^{-1} flow rate with 0.00 bar low pressure and high pressure at 400.00 bar. The separation is achieved using a linear elution gradient with mobile phases A (HPLC grade water) and B (acetonitrile) respectively. The UV/V acquisition wavelength was adjusted from 210-800 nm with following specifications; signal A 240 nm, signal B 247 nm, band/slit with 4 nm, spectra step 2.0 nm, spectra threshold 10.0 mAU, with 20min stop time. Compound identification was accomplished by comparing the retention times of the component in the HPLC chromatogram to those of the available standards chromatogram (Hossam et al., 2019; Pille-Riin et al., 2023).

Fourier Transform Infrared Spectroscopy

To observed the functional group existence, FT-IR analysis of crude extracts were performed in FT-IR spectrometer (Agilent Cary 360, USA) equipped with ATR (attenuated total reflectance) sampling unit with a resolution of $8cm^{-1}$ and scan range of 4000-650 cm^{-1} at Chemistry department, Islamia University of Bahawalpur. Dried extract samples were placed on the system window, ensure that the crystal is cleaned. Turn on the Dail Path and operate the samples at required pathlength. The generated data analysis was performed on Agilent Micro-Lab PC software (Ramya et al., 2022).

Liquid Chromatography Mass Spectrometry

The LCMS were performed using 6400 series Agilent Triple Quad LC-MS version 10.0- 127 instrument was used at the following chromatographic conditions; mobile phase A was 0.04% acetic acid, mobile phase B was acetonitrile, 5µL sample with 0.5 mL/min flow rate and 30 °C column temperature. Positive ion mode with 35 psi atomization gas pressure, 10 L/min dry gas velocity, 200 °C drying temperature and 5000 V Ionization voltage. Software implemented to handle chromatograms and mass spectra was Mass Hunter Software Suite (Xu et al., 2012).

Gas Chromatography Mass Spectrometry

GC-MS was done using Agilent GG 7890B-MSD 5977B (USA), equipped with capillary column (60m x 0.25-micron x DB-5MS). Total GC running time was 40-45 min in which 99.99% Helium was used at a constant flow of 1ml/min. The temperature programming with initial column oven temperature 50ºC-60 ºC (1 min), with an increase of 60 ºC-200 ºC (8 min) to 200 ºC-230 ºC (2 min). 1µl of each extract was injected and the injector temperature was 25ºC while for the mass spectrometer, the ion source temperature was 23ºC with an interface temperature of 30ºC and recorded over a scan range of 46 to 800m/z with electron impact ionization energy of 70 eV. Software implemented to handle chromatograms and mass spectra was Mass Hunter Software Suite. By comparing the average peak area to the overall area and matching the GC-MS results with the National Institute of Standards and Technology (NIST) library, the relative percentage quantity of each chemical was computed (Amaechi, 2021; Ramya et al., 2022).

Results

TLC of crude ethanol extract and n-Hex, DCM, EtAC fractions

The best solvent system for *A. nilotica* whole branch *n*-Hex fraction was 30 % EtAC/ *n*-Hex, where detected nine spots; for DCM fraction was 45 % EtAC/ *n*-Hex, where detected seven spots; and for EtAC fraction was 80 % EtAC/ *n*-Hex were detected 4 spots. For *A. nilotica* bark *n*-Hex fraction best solvent system was 30 % EtAC/ *n*-Hex, reveals 4 spots; DCM fraction in 45 % EtAC/ *n*-Hex system reveals in nine spots, while EtAC fraction in 80 % EtAC/ *n*-Hex

system reveals 4 spots. For *M. azedarach* leaves the best solvent system for *n*-Hex fraction was 35 % EtAC/ *n*-Hex, where detected seven spots; for DCM fraction was 2% MeOH/DCM, where detected six spots; and for EtAC fraction was 18 % MeOH/DCM, where detected seven spots.

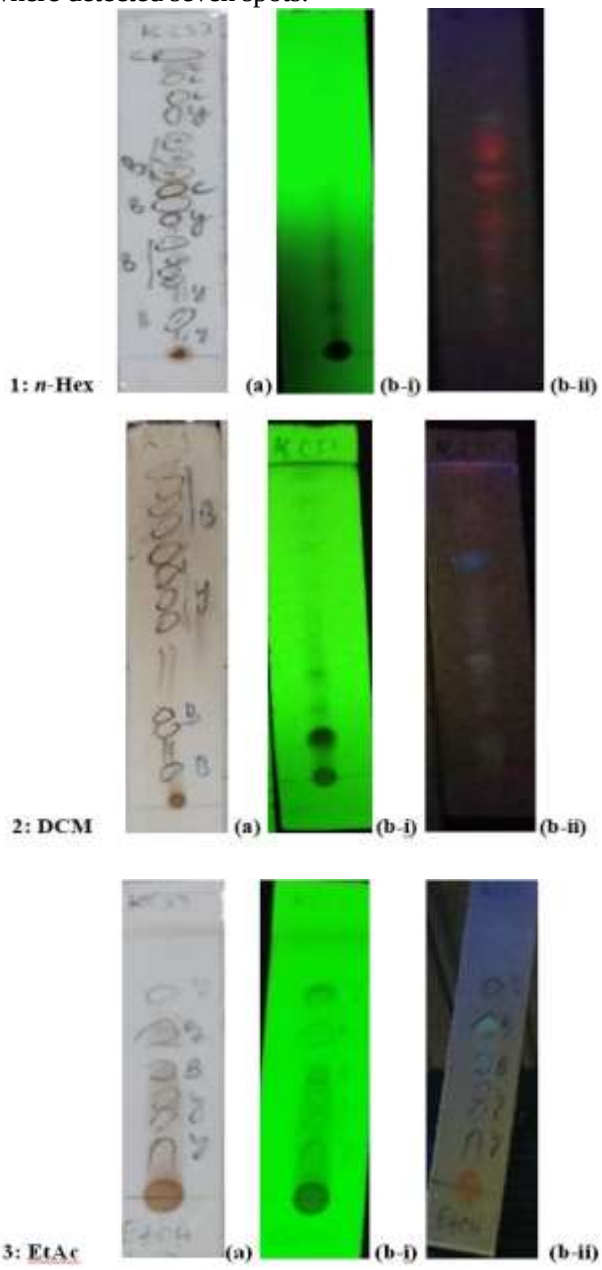


Figure-1. TLC plates of *A.nilotica* whole branch, (a) *n*-Hex, (b) DCM, (c) EtAC fractions, visualized under (a) visible light, (b) UV light at (i) 254 nm (green panel) and (ii) 365 nm (blue panel)

For *M. azedarach* bark *n*-Hex best solvent system was 40 % EA/ *n*-Hex, revealed seven spots, DCM fraction in 1% MeOH/DCM system reveals five spots, while EtAC fraction in 95 % EA/ *n*-Hex system reveals five spots**.** For *E. hirta* best solvent system for *n*-Hex fraction was10%DCM / *n*-Hex, where detected six spots; for DCM fraction was1.8% EA/MeOH, where detected two spots; and for EtAC fraction 1.8% EA+ MeOH + 2 drops Acetic Acid solvent system was best, where two spots detected**.** Color of spots were observed and *Rf* values of developed spots of different extracts were calculated at different wavelengths (Figure 1,2,3,4 & 5).

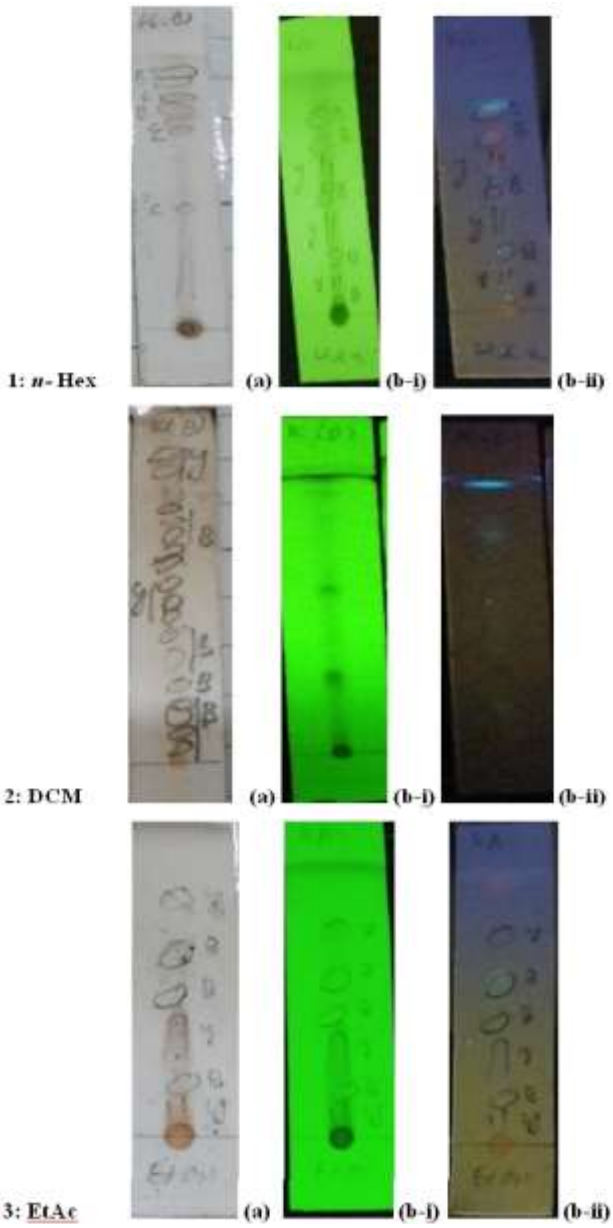


Figure-2. TLC plates of *A. nilotica* bark, (a) *n*-Hex, (b) DCM, (c) EtAC fractions, visualized under (a) visible light, (b) UV light at (i) 254 nm (green panel) and (ii) 365 nm (blue panel)

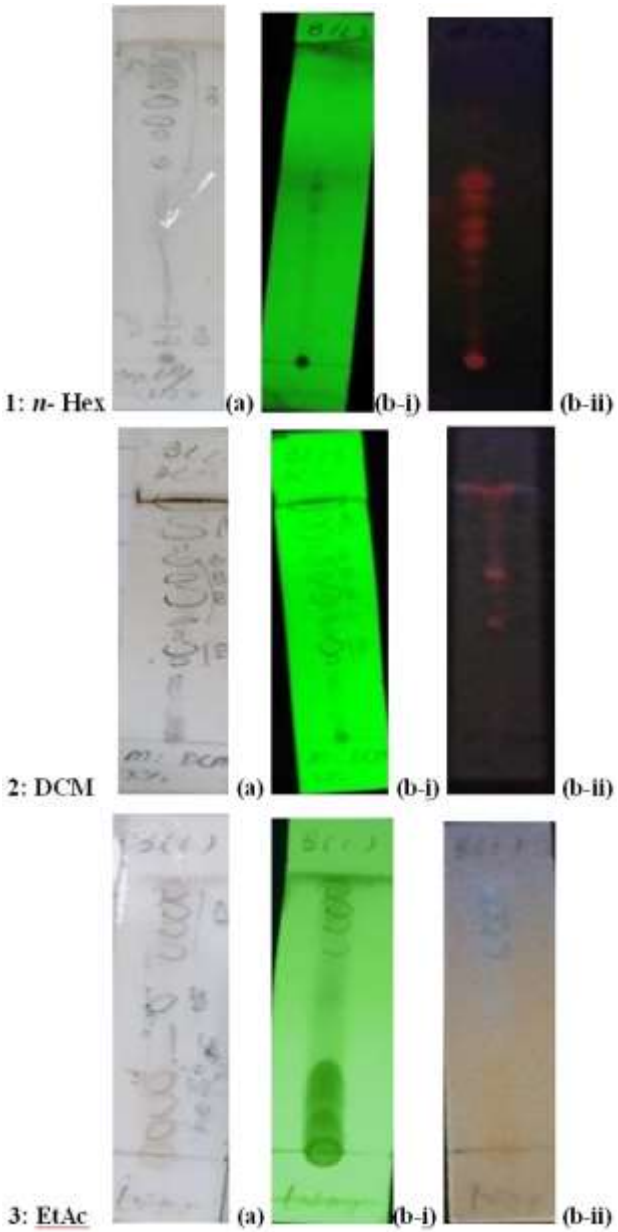


Figure-3. TLC plates of *M. azedarach* leaves, (1) *n*-Hex, (2) DCM, (3) EtAC fractions, visualized under (a) visible light, (b) UV light at (i) 254 nm (green panel) and (ii) 365 nm (blue panel)

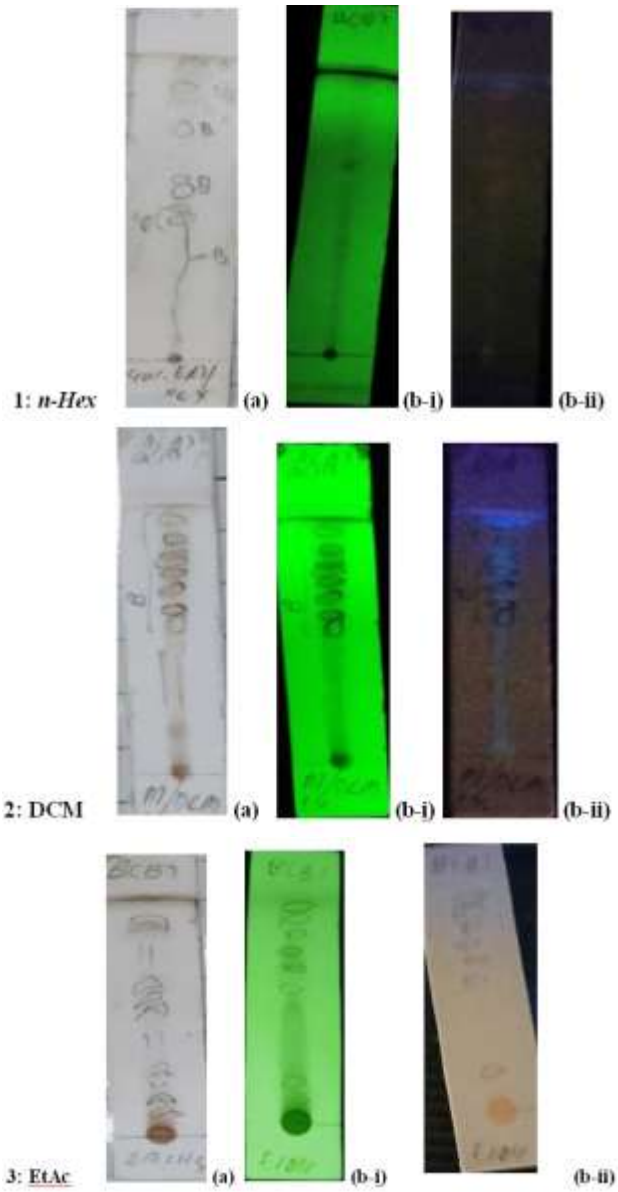


Figure-4. TLC plates of *M. azedarach* bark, (a) *n*-Hex, (b) DCM, (c) EtAC fractions, visualized under (a) visible light, (b) UV light at (i) 254 nm (green panel) and (ii) 365 nm (blue panel)

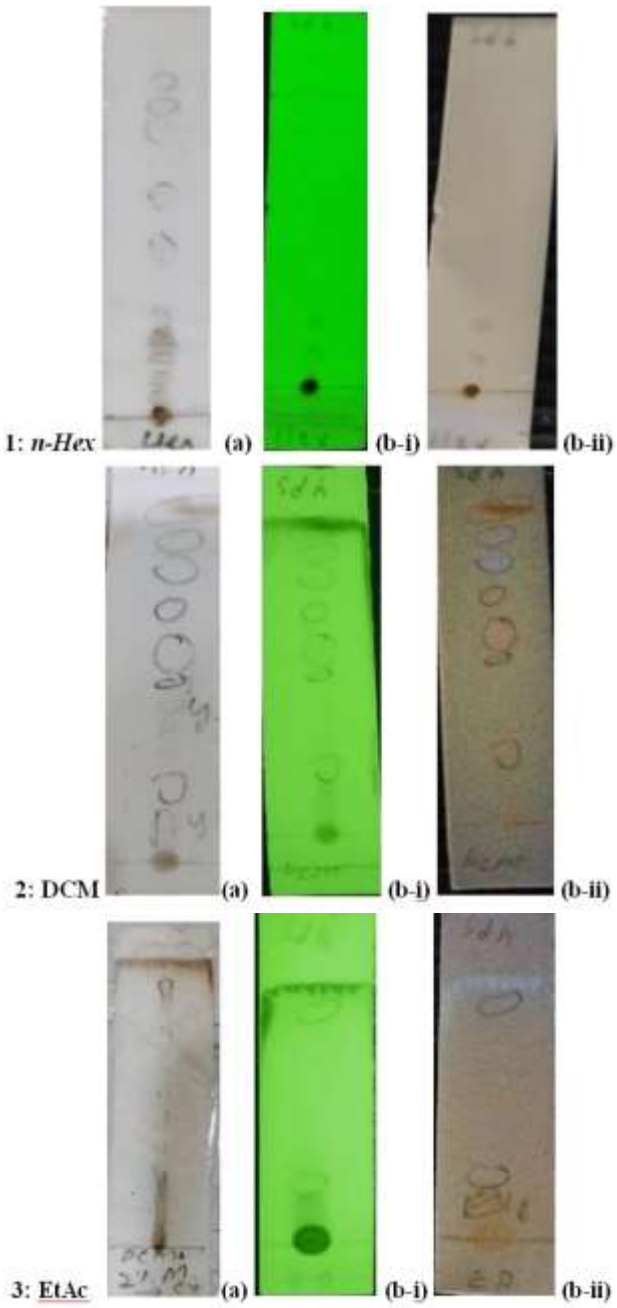


Figure-5. TLC plates of *E. hirta* whole plant, (a) *n*-Hex, (b) DCM, (c) EtAC fractions, visualized under (a) visible light, (b) UV light at (i) 254 nm (green panel) and (ii) 365 nm (blue panel)

FT-IR spectroscopy for functional group identification

The FT-IR spectra showed the characteristic prominent peaks of various functional groups of phytochemicals were observed. The IR spectra gives the broad peaks at 3263.2, 3350.8 which indicate the presence of N-H stretching. Peaks at 3201.7, 3205.5 and 3239.0 corresponds the O-H stretch. The peaks obtained at 2113.4 and 2115.2 indicated C≡C functional group whereas peaks at 1684.7, 1701.5 and 1707.1 indicated C=O stretching. The peaks at 1604.6 and 1608.3 indicated the presence of C=C stretching, whereas C=C aromatic system peaks observed at 1436.8, 1438.7, 1442.4 and 1457.3. The presence of NO_2 group was confirmed at 1317.6, 1321.3, 1364.2, 1375.3, 1518.8 and 1520.7 peaks. Similarly, C-F group showed the peaks at 1030.6, 1034.3, 1036.1, 1045.5, 1103.3, 1105.2, 1157.3, 1196.5, 1198.3, 1202.0, 1243.0. More ever the peak at 989.6 hinted the presence of $RCH{=}CH_2$ mono-substituted alkene group whereas peak at 818.1may be due to C-Cl stretch. The peaks at 866.60 indicated =C-H bend whereas the peaks at 2853.2, 2924.0 indicated the C- H functional group. The maximum number of functional groups were observed in *M. azedarach* bark extract, followed by *A. nilotica* stem/bark extract and *M. azedarach* stem extract while the least number of functional group were observed in *E. hirta* extract. (Figure 6 a, b, c, d & e).

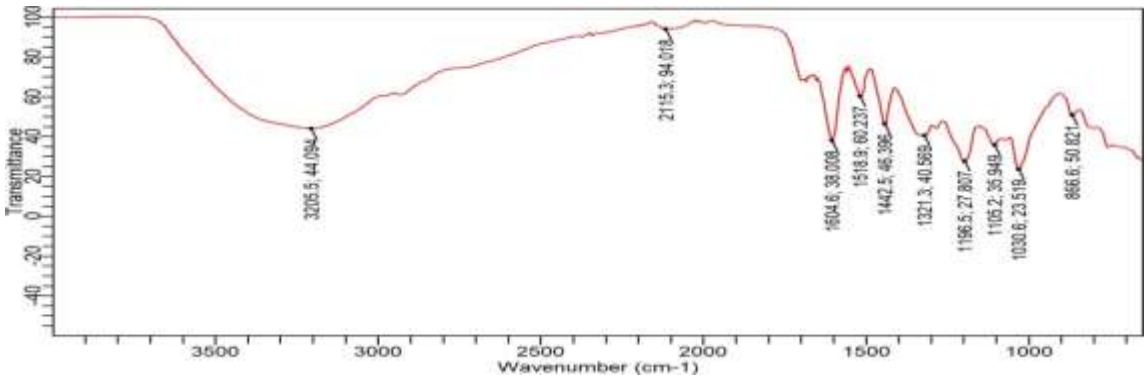
High-performance liquid chromatography for identification & quantification of compounds

The HPLC chromatogram of *A. nilotica* stem extract revealed seven compounds with retention time 4.240 --16.926 including 0.0238µg/mg gallic acid, 0.0201µg/mg *p*-hydroxy benzoic acid, 0.0430µg/mg gentisic acid, 0.2395µg/mg protocatechuic acid, 0.0221µg/mg catechin, 0.0221µg/mg syringic acid and 0.0221µg/mg chlorogenic acid. The extract of *A. nilotica* bark also revealed the presence of 8 compounds with retention time 4.380 --18.574 including 0.2099µg/mg gallic acid, 0.1890µg/mg protocatechuic acid, 0.0637µg/mg *p*-hydroxy benzoic acid, 0.1840µg/mg gentisic acid, 0.0483µg/mg vanillic acid, 0.0308µg/mg catechin, 0.0371µg/mg syringic acid and 0.1382µg/mg epi-catechin.

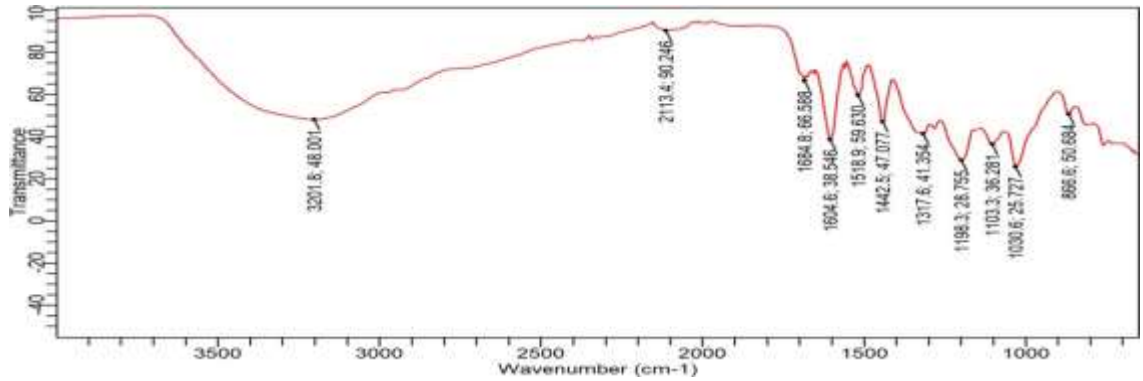
The HPLC chromatogram of *M. azedarach* leaves extract revealed the presence of 22 compounds with retention time of 1.444 --19.112, including 0.5996µg/mg quercetin, 45.6725µg/mg hydroxy ferulic acid hexoside, 25.5353µg/mg rutin, 11.3697µg/mg vanillic acid, 0.4004µg/mg ferulic acid, 2.0330µg/mg ferulic acid hexoside II, 0.1681µg/mg feruloylquinic acid, 1.3451µg/mg myricetin hexoside, 3.6335µg/mg kaempferol -3- *O* -rutinoside, 1.4663µg/mg kaempferol -3- *O* – rhamnoside, 0.4112µg/mg procyanidin dimer B, 0.1888µg/mg toosendanin, 1.0653µg/mg quercetin-7-

O- glycoside, 0.2385µg/mg kaempferol, 0.2896µg/mg catechin-7-O- glycoside, 0.0588 µg/mg apigenin -7-*O*- glycoside, 0.3530µg/mg kaempferol -7-*O*-glycoside, 0.0776µg/mg catechin-5-*O*-glycoside, 0.7655µg/mg capric acid methyl ester, 0.1138µg/mg 8- hexadecene, 01225µg/mg phytadiene, 0.0191µg/mg *γ*-*n*-Amyl butyrolactone. The extract of *M. azedarach* bark also revealed the presence of eleven compounds with retention time of 2.901--16.161, including 1.9939µg/mg rutin, 0.0336µg/mg apigenin, 0.1268µg/mg luteolin, 0.0442µg/mg kaempferol, 0.0116µg/mg kaempferol -3-*O*-glycoside, 0.0641µg/mg quercetin -7-*O*-glycoside, 0.0519µg/mg apigenin-7-O- glycoside, 5.3490µg/mg kaempferol -7-*O*-glycoside, 0.0518µg/mg catechin-5-*O*-glycoside, 0.0441µg/mg capric acid methyl ester and 0.0832µg/mg 8-hexadecene**.**

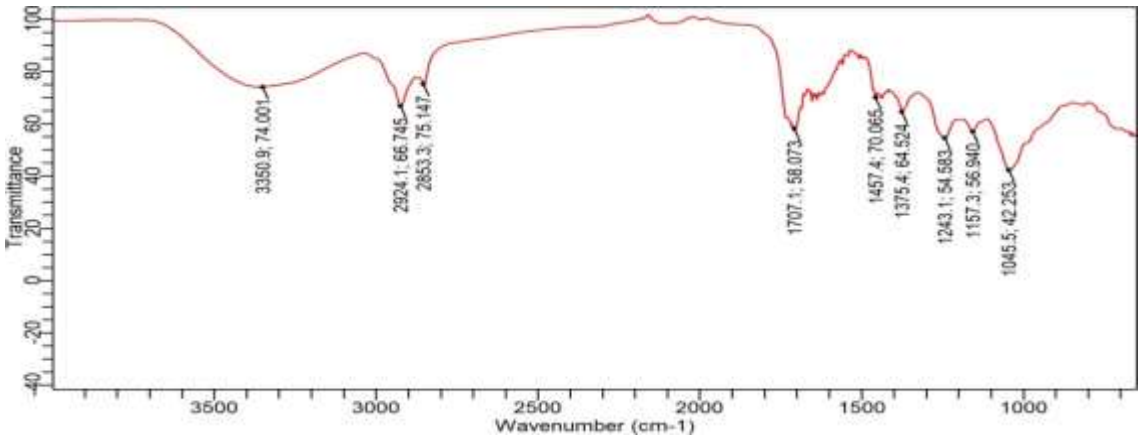
In the present study the HPLC analysis of ethanolic extract of *E. hirta* whole plant showed the presence of seven compounds with retention time of 2.408 --11.138, including 32.5482µg/mg rutin, 1.0282µg/mg gallic acid, 0.2190µg/mg tannic acid, 0.8410µg/mg resorcinol, 0.0955µg/mg ellagic acid, 0.0854µg/mg benzoic acid and 0.5838µg/mg quercetin. Chromatograms of extracts are shown in Figure-7 (a, b, c, d, e) and Table 1.



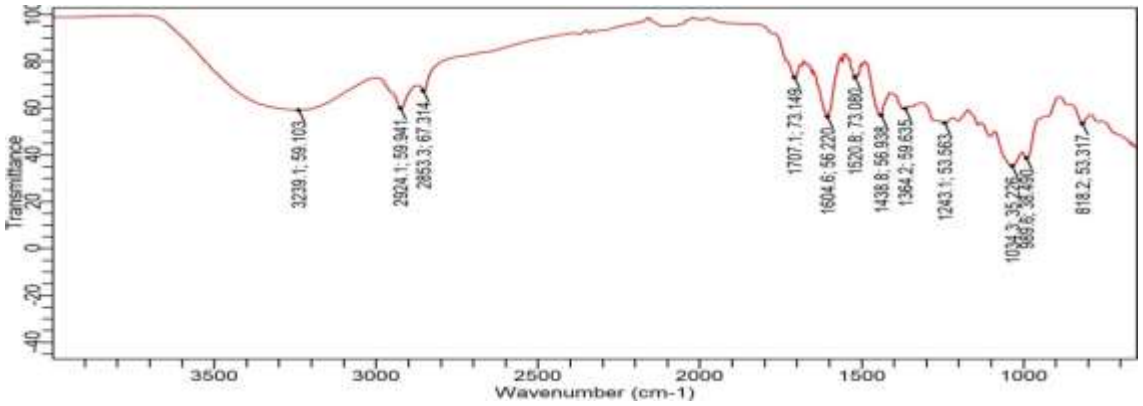
(a) ***A. nilotica* Whole branch**



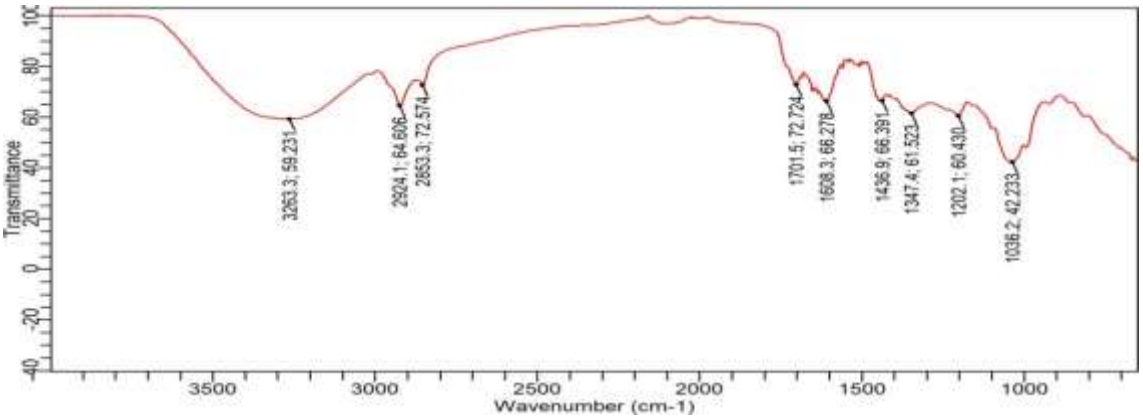
(b) ***A. nilotica Bark***



(c) ***M. azedarach*** Leaves



(d) ***M. azedarach*** **Bark**



(e) ***E. hirta*** **whole plant**

Figure-6: **FT-IR spectrums representing potential peaks in ethanolic extracts of medicinal plants (a) *A. nilotica* Stem (b) *A. nilotica* Bark (c) *M. azedarach* leaves (d) *M. azedarach* bark (e) *E. hirta* whole plant**

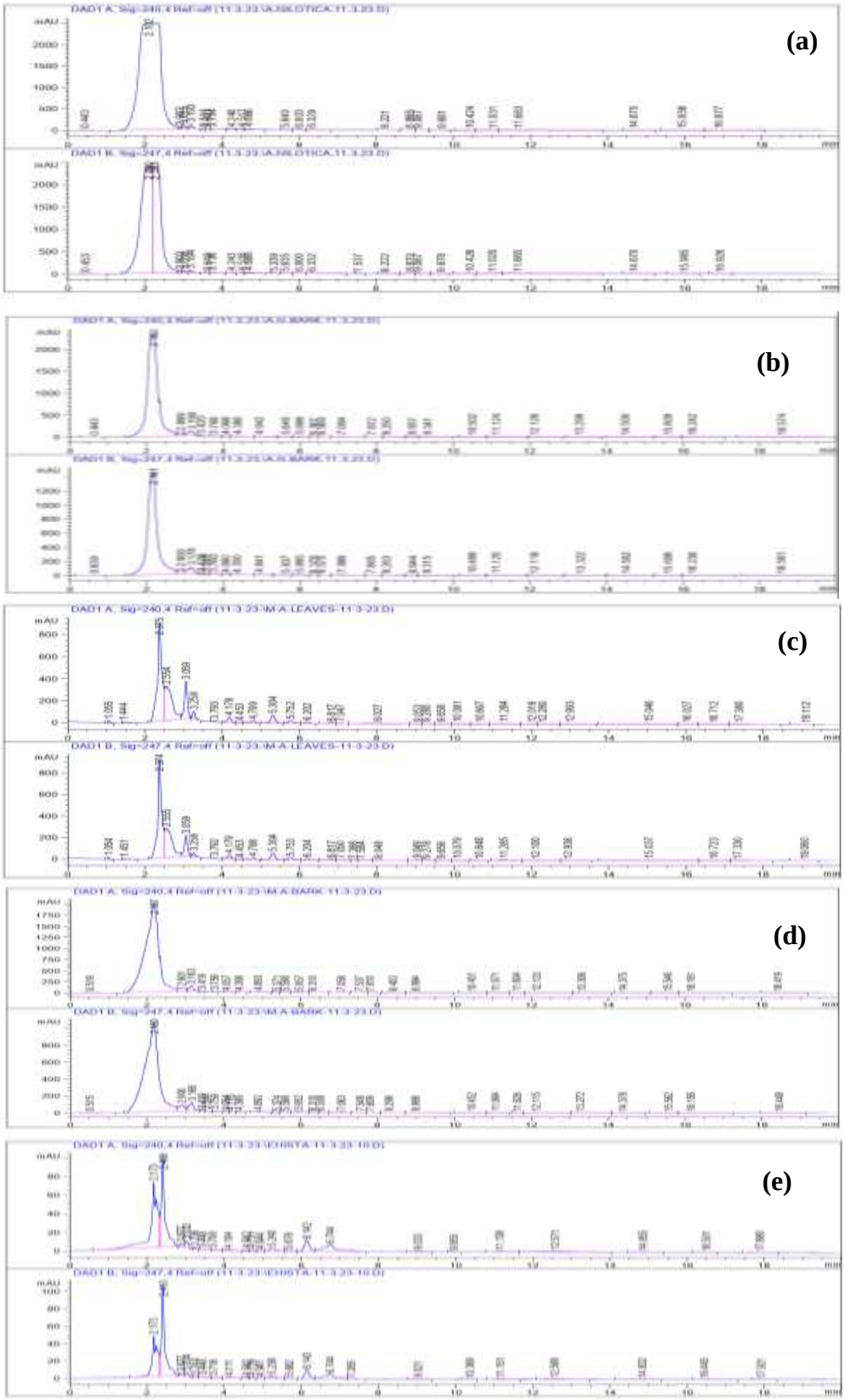


Figure-7. HPLC chromatographs (a) *A. nilotica* Stem (b) *A. nilotica* Bark (c) *M. Azedarach* Leaves (d) *M. Azedarach* Bark (e) *E. hirta* whole plant

Table-1. HPLC quantifications of ethanolic extract of selected Cholistan medicinal plants.

	RT (min)	Compound Identified	Molecular Formula	Molecular Weight (g/mol)
A. nilotica whole branch	4.240	Gallic acid	$C_7H_6O_5$	170.12
	9.691	*p*-hydroxy benzoic acid	$C_7H_6O_3$	138.12
	11.031	Gentisic acid	$C_7H_6O_4$	154.12
	11.683	Protocatechuic acid	$C_7H_6O_4$	154.12
	15.938	Catechin	$C_{15}H_{14}O_6$	290.27
	16.877	Syringic acid	$C_9H_{10}O_5$	198.17
	16.926	Chlorogenic acid	$C_{16}H_{18}O_9$	354.31
A. nilotica **Bark**	4.380	Gallic acid	$C_7H_6O_5$	170.12
	7.084	Protocatechuic acid	$C_7H_6O_4$	154.12
	9.341	*p*-hydroxy benzoic acid	$C_7H_6O_3$	138.12
	11.124	Gentisic acid	$C_7H_6O_4$	154.12
	12.126	Vanillic acid	$C_8H_8O_4$	168.15
	15.609	Catechin	$C_{15}H_{14}O_6$	290.27
	16.242	Syringic acid	$C_9H_{10}O_5$	198.17
	18.574	Epi-catechin	$C_{15}H_{14}O_6$	290.27
M. azedarach **Leaves**	1.444	Quercetin	$C_{15}H_{10}O_7$	302.23
	2.375	Hydroxy ferulic acid hexoside	$C_{10}H_{10}O_5$	210.18
	2.554	Rutin	$C_{27}H_{30}O_{16}$	610.50
	3.059	Vanillic acid	$C_8H_8O_4$	168.15
	3.793	Ferulic acid	$C_{10}H_{10}O_4$	194.18
	4.178	Ferulic acid hexoside II	$C_{10}H_{10}O_5$	210.18
	4.453	Feruloylquinic acid	$C_{17}H_{20}O_9$	368.30
	4.799	Myricetin hexoside	$C_{28}H_{32}O_{17}$	640.50
	5.304	Kaempferol -3- *O* -rutinoside	$C_{33}H_{40}O_{20}$	756.70
	5.752	Kaempferol -3- *O* - rhamnoside	$C_{33}H_{40}O_{18}$	724.70
	6.202	Quercetin	$C_{15}H_{10}O_7$	302.23
	6.817	Procyanidin dimer B	$C_{30}H_{26}O_{12}$	578.50
	7.047	Toosendanin	$C_{30}H_{38}O_{11}$	574.60
	8.027	Quercetin-7-*O*- glycoside	$C_{21}H_{20}O_{12}$	464.40
	9.280	Kaempferol	$C_{15}H_{10}O_6$	286.24
	9.658	Catechin-7-*O*- glycoside	$C_{21}H_{24}O_{11}$	452.40
	10.667	Apigenin -7-*O*- glycoside	$C_{30}H_{26}O_{12}$	578.50
	11.284	Kaempferol -7-*O*-glycoside	$C_{21}H_{20}O_{11}$	448.40
	12.993	Catechin-5-*O*- glycoside	$C_{21}H_{24}O_{11}$	452.40
	15.046	Capric acid methyl ester	$C_{11}H_{22}O_2$	186.29
	16.027	8- Hexadecene	$C_{16}H_{32}$	224.42
	17.380	Phytadiene	$C_{20}H_{38}$	278.50
	19.112	γ-*n*-Amyl butyrolactone	$C_9H_{16}O_2$	156.22
M. azedarach **Bark**	2.901	Rutin	$C_{27}H_{30}O_{16}$	610.50
	3.759	Apigenin	$C_{15}H_{10}O_5$	270.24
	4.893	Luteolin	$C_{15}H_{10}O_6$	286.24
	6.310	Kaempferol	$C_{15}H_{10}O_6$	286.24
	7.810	Kaempferol -3-*O*-glycoside	$C_{21}H_{20}O_{11}$	448.40
	8.994	Quercetin -7-*O*-glycoside	$C_{21}H_{20}O_{12}$	464.40
	10.451	Apigenin-7-*O*- glycoside	$C_{30}H_{26}O_{12}$	578.50
	11.604	Kaempferol -7-*O*-glycoside	$C_{21}H_{20}O_{11}$	448.40
	12.133	Catechin-5-*O*-Glycoside	$C_{21}H_{24}O_{11}$	452.40
	15.546	Capric acid methyl ester	$C_{11}H_{22}O_2$	186.29
	16.161	8-Hexadecene	$C_{16}H_{32}$	224.42
E. hirta	2.408	Rutin	$C_{27}H_{30}O_{16}$	610.50
	2.877	Gallic acid	$C_7H_6O_5$	170.12

	4.944	Tannic acid	$C_{76}H_{52}O_{46}$	1701.2
	6.744	Gallic acid	$C_7H_6O_5$	170.12
	7.285	Resorcinol	$C_6H_6O_2$	110.11
	9.955	Ellagic acid	$C_{14}H_6O_8$	302.19
	10.369	Benzoic acid	$C_7H_6O_2$	122.12
	11.138	Quercetin	$C_{15}H_{10}O_7$	302.23

LCMS analysis for identification of compounds

The LCMS spectrum of *A. nilotica* stem extract confirmed the presence of 12 compounds with 1.612 -11.183 retention time including; 100.0µg/mg kaempferol, 56.27µg/mg epicatechin-5-gallate, 1.90µg/mg gallocatechin 7,4'-di-*O*- gallate, 4.06µg/mg caffeic acid hexose, 14.12µg/mg 1,6, di-*O*-galloyl-*β*-*O*- glucopyranose, 3.17µg/mg gallic acid, 1.14µg/mg *L*-arabinose, 2.45µg/mg magniferin, 21.44µg/mg digallocatechin-5-gallate, 2.23µg/mg 1,3, di-*O*-galloyl-*β*-*O*- glucopyranose, 1.11µg/mg monogalloyal glucose and 1.42µg/mg dialloyal glucose. The LCMS scan identified fifteen compounds in *A. nilotica* bark extract with 0.700 - 14.202 retention time including; 65.43µg/mg ethyl gallate, 100.00µg/mg kaempferol, 82.69µg/mg myricetin, 19.78µg/mg monogalloyal glucose, 11.83µg/mg digallocatechin-5-gallate, 47.35µg/mg vitexin, 40.66µg/mg caffeic acid hexose, 15.81µg/mg dialloyal glucose, 41.83µg/mg magniferin, 4.66µg/mg *L*-arabinose, 2.04µg/mg epicatechin, 86.55µg/mg gallic acid, 4.15µg/mg toxifolin, 6.12µg/mg catechi-7,4'-di-*O-gallate*, 1.84µg/mg catechin-7,3'-di-gallate. In *M. azedarach* leaves extract eleven compounds identified with 1.494 - 13.031 retention time. The identified compounds were 76.04µg/mg quercetin, 22.02µg/mg quercetin 3-*O*-[2-O-6-*Z*-*P*-coumaroyl- glucopyranoside]-rhamnoside, 8.01µg/mg nicotiflorin, 21.06µg/mg soyacerebroside I, 4.711µg/mg quercetin-7-*O*-*β*-*D*-glucopyranoside, 3.51µg/mg salannal, 14.37µg/mg quercetin-3-*O*-(2",6"-digalloyl)-*β*-*D*-galactopyranoside, 16.58µg/mg meliarachin, 1.04µg/mg methyl (23 *S*)-, 80.21µg/mg toosendanin derivates and 62.06µg/mg salannin. 14 compounds identified in *M. azedarach* bark extract with 1.659 - 13.039 retention time including; 42.04µg/mg quercetin, 1.27µg/mg quercetin 3-*O*-[2"-*O*-(6"'-*O*-*P*-coumaroyl)-*β*-*D*- glucopyranoyl]-*α*-*L*-rhamnopyranoside, 100.0µg/mg nicotiflorin, 32.31µg/mg isosakuraneti-7-*O*-neohesperidoside, 18.20µg/mg (8,8 dimethyl-2,10-dioxo-9*H*-pyrano[2,3-f] chromen-9-yl) (*Z*)-2-methylbut-2-enoate, 2.65µg/mg strophanthidine, 13.67µg/mg fatty acid 18:4, 9.64µg/mg salannal, 16.88µg/mg soyasaponin, 2.93µg/mg quercetin 3-*O*-*(*2",6'-digalloyl)-*β*-D-galactopyranoside, 15.52µg/mg meliarachin, 3.74µg/mg toosendanin, 17.32µg/mg toosendanin derivates and 49.95µg/mg salannin**.** The LCMS analysis of *E. hirta* whole plant extract revealed eleven compounds with 1.557 - 10.884 retention time. The identified compounds were; 100.00µg/mg quinic acid, 29.12µg/mg triterpenoids, 4.54µg/mg neochlorogenic acid, 8.99µg/mg ascorbic acid, 3.97µg/mg caffeic acid, 1.43µg/mg ubmelliferone, 11.83µg/mg gallic acid, 2.82µg/mg quercetin, 1.05µg/mg chlorogenic acid, 1.23µg/mg astragalin and 4.09µg/mg syringic acid respectively (Figure 8 a, b, c, d, e & Table 2).

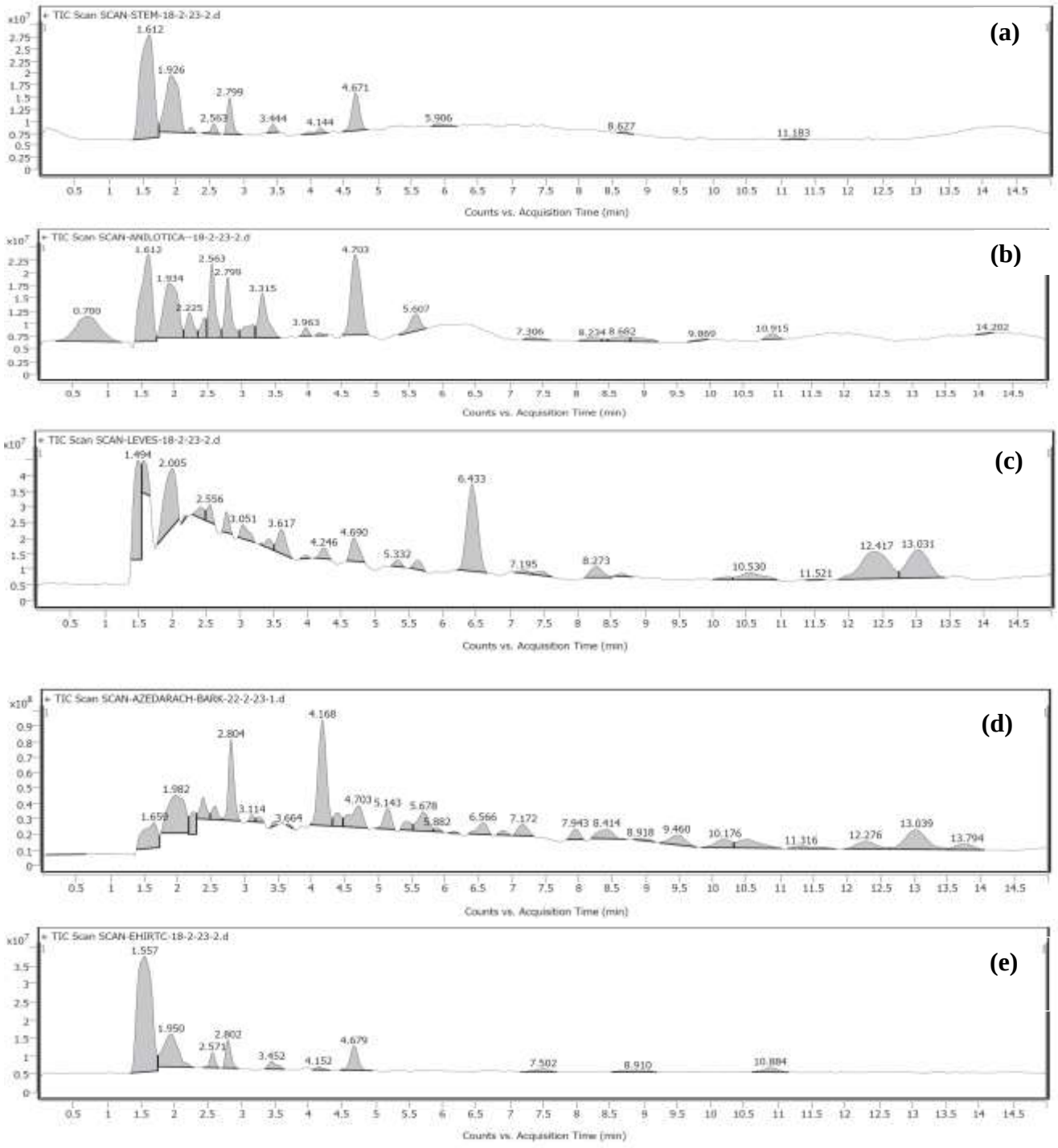


Figure-8. LCMS chromatographs (a) *A. nilotica* Stem (b) *A. nilotica* Bark (c) *M. Azedarach* Leaves (d) *M. Azedarach* Bark (e) *E. hirta* whole plant.

Table-2. LCMS analysis of ethanolic extracts of selected Cholistan medicinal plants.

Plant Extract	RT (min)	Compound Identified	Molecular Formula	Molecular Weight(g/mol)
***A. nilotica* whole branch**	1.612	Kaempferol	$C_{15}H_{10}O_6$	286.24
	1.926	Epicatechin-5-gallate	$C_{22}H_{18}O_{10}$	442.40
	2.225	Gallocatechin 7,4'-di-*O*- gallate	$C_{29}H_{22}O_{15}$	610.50
	2.563	Caffeic acid hexose	$C_9H_8O_4$	180.16
	2.799	1,6, Di-*O*-galloyl-*β*-*O*- glucopyranose	$C_{20}H_{20}O_{14}$	484.40
	3.444	Gallic acid	$C_7H_6O_5$	170.12
	3.979	*L*-Arabinose	$C_5H_{10}O_5$	150.13
	4.144	Magniferin	$C_{19}H_{18}O_{11}$	422.30
	4.671	Digallocatechin-5-gallate	$C_{22}H_{18}O_{11}$	458.40
	5.906	1,3, Di-*O*-galloyl-*β*-*O*- glucopyranose	$C_{27}H_{24}O_{18}$	636.50
	8.627	Monogalloyal glucose	$C_{13}H_{16}O_{10}$	332.26
	11.183	Dialloyal glucose	$C_{13}H_{16}O_{10}$	332.26
***A. nilotica* Bark**	0.700	Ethyl gallate	$C_9H_{10}O_5$	198.17
	1.612	Kaempferol	$C_{15}H_{10}O_6$	286.24
	1.934	Myricetin	$C_{15}H_{10}O_8$	318.23
	2.225	Monogalloyal glucose	$C_{13}H_{16}O_{10}$	332.26
	2.453	Digallocatechin-5-gallate	$C_{22}H_{18}O_{11}$	458.40
	2.563	Vitexin	$C_{21}H_{20}O_{10}$	432.40
	2.799	Caffeic acid hexose	$C_9H_8O_4$	180.16
	3.169	Dialloyal glucose	$C_{13}H_{16}O_{10}$	332.26
	3.315	Magniferin	$C_{19}H_{18}O_{11}$	422.30
	3.963	*L*-Arabinose	$C_5H_{10}O_5$	150.13
	4.168	Epicatechin	$C_{15}H_{14}O_6$	290.27
	4.503	Gallic acid	$C_7H_6O_5$	170.12
	7.306	Toxifolin	$C_{15}H_{12}O_7$	304.25
	10.915	Catechi-7,4'-di-*O*-gallate	$C_{29}H_{22}O_{14}$	594.50
	14.202	Catechin-7,3'-di-gallate	$C_{29}H_{22}O_{14}$	594.50
***M. azedarach* Leaves**	1.494	Quercetin	$C_{15}H_{10}O_7$	302.04
	3.617	Quercetin 3-*O*- [2-O-6-*Z*-*P*-Coumaroyl- glucopyranoside]-rhamnoside	$C_{36}H_{36}O_{18}$	756.19
	4.246	Nicotiflorin	$C2_7H_{30}O_{15}$	594.15
	4.690	Soyacerebroside I	$C_{40}H_{75}NO_9$	713.54
	5.332	Quercetin-7-*O*-*β*-*D*- glucopyranoside	$C_{21}H_{20}O_{12}$	464.40
	7.195	Salannal	$C_{34}H_{44}O_{10}$	612.716
	8.273	Quercetin-3-*O*-(2",6"-digalloyl)-*β*-*D*-galactopyranoside	$C_{38}H_{34}O_{17}$	762.70
	10.53	Meliarachin	$C_{30}H_{36}O_{11}$	572.60
	11.521	Methyl (23 *S*)-	$C_{25}H_{42}O_5$	422.59
	12.417	Toosendanin derivates	[M-H2O +H]+	557.40
	13.031	Salannin	$C_{34}H_{44}O_9$	596.70
***M. azedarach* Bark**	1.659	Quercetin	$C_{15}H_{10}O_7$	302.04
	3.664	Quercetin 3-*O*-[2"-*O*-(6"'-*O*-*P*-coumaroyl)-*β*-*D*- glucopyranoyl]-α-*L*-rhamnopyranoside	$C_{36}H_{36}O_{18}$	756.19
	4.168	Nicotiflorin	$C_{27}H_{30}O_{15}$	594.15
	4.703	Isosakuraneti-7-*O*-neohesperidoside	$C_{28}H_{34}O_{14}$	594.19
	5.143	(8,8 dimethyl-2,10-dioxo-9*H*-Pyrano[2,3-f] chromen-9-yl) (*Z*)-2-methylbut-2-enoate	$C_{19}H_{18}O_6$	342.11
	5.882	Strophanthidine	$C_{23}H_{32}O_6$	404.21

	6.566	Fatty acid 18:4	$C_{18}H_{28}O_4$	308.19
	7.943	Salannal	$C_{34}H_{44}O_{10}$	612.716
	8.414	Soyasaponin	$C_{48}H78O_{18}$	942.51
	8.918	Quercetin 3-*O*-(2",6'-digalloyl)-*β*-*D*-galactopyranoside	$C_{38}H_{34}O_{17}$	762.70
	10.176	Meliarachin	$C_{30}H_{36}O_{11}$	572.60
	11.316	Toosendanin	$C_{30}H_{38}O_{11}$	574.60
	12.276	Toosendanin derivates	[M-H +H] +	557.40
	13.039	Salannin	$C_{34}H_{44}O_9$	596.70
E. hirta	1.557	Quinic acid	$C_7H_{12}O_6$	192.17
	1.950	Triterpenoids	$C_{29}H_{44}O_5$	472.70
	2.571	Neochlorogenic acid	$C_{16}H_{18}O_9$	354.09
	2.802	Ascorbic acid	$C_6H_8O_6$	MW
	3.452	Caffeic acid	$C_9H_8O_4$	180.04
	4.152	Ubmelliferone	$C_9H_6O_3$	162.14
	4.679	Gallic acid	$C_7H_6O_5$	170.12
	7.502	Quercetin	$C_{15}H_{10}O_7$	302.04
	8.705	Chlorogenic acid	$C_{16}H_{18}O_9$	354.31
	8.910	Astragalin	$C_{21}H_{20}O_{11}$	448.10
	10.884	Syringic acid	$C_9H_{10}O_5$	198.17

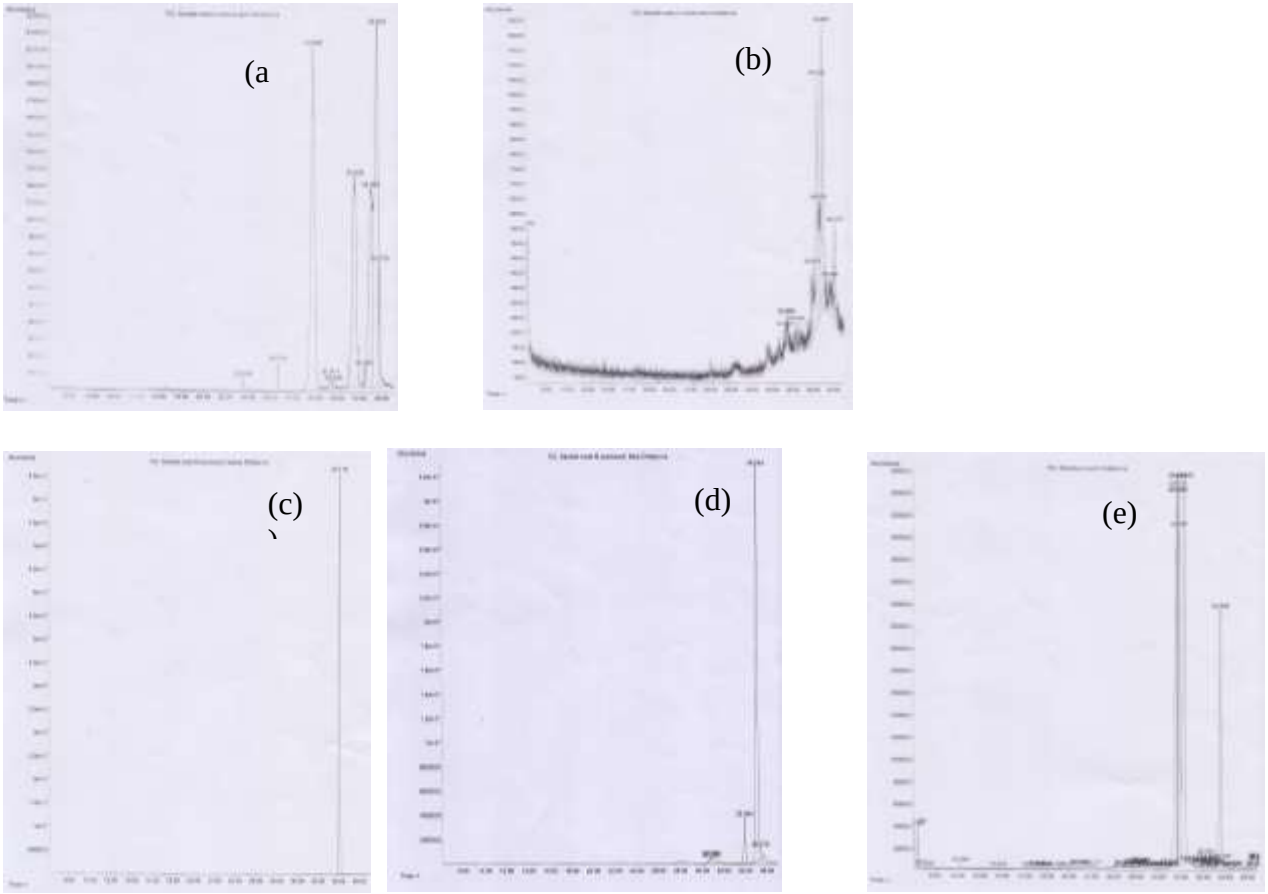


Figure-9: GCMS chromatographs (a) *A. nilotica* Stem (b) *A. nilotica* Bark (c) *M. Azedarach* Leaves (d) *M. Azedarach* Bark (e) *E. hirta* whole plant.

Table-3: GCMS analysis of ethanolic extracts of selected Cholistan medicinal plants

Plant Extract	RT (min)	Compound Identified	Molecular Formula	Molecular Weight (g/mol)
***A. nilotica* Stem**	23.529	2-Ethyl-1-butanol, trifluoroacetate	$C_8H_{13}F_3O_2$	198.18
	26.705	2-Methoxydecanoic acid	$C_{11}H_{22}O_3$	202.29
	29.690	Cyclohexane carboxamide	$C_7H_{13}NO$	127.18
	31.411	1,4- Octadiene	C_8H_{14}	110.1968
	31.648	Cyclodecene	$C_{10}H_{18}$	138.2499
	33.522	*Z, Z*-6,13-Octadecadien-1-ol acetate	$C_{20}H_{36}O_2$	308.50
	34.399	Bicyclo [3.3.1] nonae	C_9H_{16}	124.2233
	34.960	4,7-Octadecadienoic acid, methyl ester	$C1_9H_{34}O_2$	294.50
	35.416	9,12- Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.4721
	35.779	Tricyclo [4.4.0.0 (3,9)] decane	C10H16	136.23
***A. nilotica* Bark**	6.180	8-Methyl-6-nonenoic acid	$C_{10}H_{18}O_2$	170.25
	31.350	Acetic acid, trifluoro-, undecyl ester	$C_{13}H_{23}O_2$	268.3157
	31.512	Trichloroacetic acid, tetradecyl ester	$C_{16}H_{29}Cl_3O_2$	359.80
	32.403	Trichloroacetic acid, hexadecyl ester	$C_{18}H_{33}CL_3O_2$	387.812
	33.974	2-Octyne	$C_{18}H_{14}$	110.1968
	34.430	4-Methyl-1-hepten-4-ol acetate	$C_{10}H_{18}O_2$	170.25
	34.579	1,4-Octadiene	C_8H_{14}	110.1968
	34.961	1-Methyl -1,2,3,6-tetrahydropyridin	$C_6H_{11}N$	97.1582
	36.157	3-Octyne	C_8H_{14}	110.1968
***M. azedarach* Leaves**	34.116	11- Cyclohexyldimethylsilyloxyundec-1-ene	$C_{19}H_{38}OSi$	310.60
***M. azedarach* Bark**	30.740	3-Cyclohexene-1-ethanol, alpha. -ethenyl -. Alpha., 3-dimethyl-6-(1-methylethylidene)-	C15H24O	220.3505
	30.936	Cyclopropane,1,1'-ethenylidenebis	C8H12	108.1809
	33.944	1,4-Cyclotadiene	C8H12	108.1809
	35.144	6-Octadecnoic acid, methyl ester, (*Z*)-	C19H36O2	296.4879
	35.379	*N*- (p-Methoxyphenyl) -*N'* – (1,1-dicyanoethyl)-diazene	$C_{11}H_{10}N_4O$	214.22
E. hirta	6.411	1-Methylcyclopropanecarboxylic acid-2-Pentyn-1-ol	$C_5H_8O_2$	100.1158
	6.486	Zinc, bis(3-methyl-2-butenyl)-	$C_{15}H_{20}N_2OS_2$	308.50
	10.264	Furan, 2-butyltetrahydro-	$C_{18}H_{16}O$	128.2120
	13.610	2-Pyridinecarboxylic acid, 3-nitro-, methyl ester	$C_7H_6N_2O_4$	182.13
	17.120	5-Pyrimidinecarboxylic acid, hexahydro-5-(1-methylethyl)-2,4,6-trioxo-	$C_8H_{10}N_2O_5$	214.18
	17.617	Furan,2,3-dihydro-5-methyl	C_5H_8O	84.1164
	24.895	12-Octadecenal	$C_{18}H_{34}O$	266.50
	25.597	2*H*-Pyran,2-(3-butynyloxy) tetrahydro-	$C_9H_{14}O_2$	154.2063
	25.855	2-(1-Cyclohexenyl) ethylamine	$C_8H_{15}N$	125.2114
	26.289	Cyclopropane carboxylic acid,2-methylene-, methyl ester	$C_6H_8O_2$	112.1265
	26.375	9-Hexadecenoic acid, methyl ester, (*Z*)	$C_{17}H_{32}O_2$	268.4348
	26.442	7-Tetradecane	$C_{14}H_{28}$	196.3721
	26.507	*N*-Octanoic acid isopropyl ester	$C_{11}H_{22}O_2$	186.2912
	26.959	2*H*-Pyran,2-(3-butynyloxy) tetrahydro-	$C_9H_{14}O_2$	154.2063
	27.273	2*H*-Pyran,3,6-dihydro-4-methyl-2-(2-methyl-1-propenyl)-	$C_{10}H_{16}O$	152.2334
	28.567	2,6-Dimethyl-8-oxoocta-2,6-dienoic acid,	$C_{11}H_{16}O_3$	196.24

		methyl ester		
	28.962	Cyclopropane carboxylic acid, nonyl ester	$C_{13}H_{24}O_2$	212.33
	29.631	Nonadecanoic acid	$C_{19}H_{38}O_2$	298.5038
	29.665	2-Trimethylsilyoxyoct-3-ene	--	--
	29.711	4*H*-1-Benzopyran-4-one,5-hydroxy-7-methoxy-2-(4-methoxyphenyl)-	$C1_7H_{14}O_5$	298.2901
	30.249	10-Oxocyclodec-2-enecarboxylic acid, methyl ester	$C_{12}H_{18}O_3$	210.27
	32.183	Heptanedioc acid,3-methyl-, dimethyl ester	$C_{10}H_{18}O_4$	202.2475
	32.51	Tridecanoic acid	$C_{13}H_{26}O_2$	214.3443
	33.050	4H-1Benzothiopyran-4-one, octahydro-2-methyl-,1,1-dioxide	--	--
	33.506	1-Trimethylsilyloxy-3,4-dimethylcyclohexane	$C_{11}H_{24}OSi$	200.39
	36.889	Oleic acid	$C_{18}H_{34}O_2$	282.4614
	36.917	16-Hydroxyhexadecanoic acid	$C_{16}H_{32}O_3$	272.4235
	36.933	Heneicosanoic acid	$C_{21}H_{42}O_2$	326.5570

GCMS analysis for identification and structure elucidations of compounds

In the present study a total of ten compounds were detected in *A. nilotica* stem extract with 23.529 - 35.779 retention time including; 0.140µg/mg 2-ethyl-1-butanol, trifluoroacetate, 0.289µg/mg 2-methoxydecanoic acid, 30.822µg/mg cyclohexane carboxamide, 0.674µg/mg 1,4- octadiene, 0.276µg/mg cyclodecene, 19.213µg/mg *Z, Z*-6,13-octadecadien-1-ol acetate, 0.284µg/mg bicyclo [3.3.1] nonae, 15.782µg/mg 4,7-octadecadienoic acid methyl ester, 29.836µg/mg 9,12- octadecadienoic acid, methyl ester and 2.683µg/mg tricyclo [4.4.0.0 (3,9)] decane; while in *A. nilotica* bark extract with 6.180 - 36.157 retention time including; 4.465µg/mg 8-methyl-6-nonenoic acid, 1.864µg/mg acetic acid, trifluoro-, undecyl ester, 0.889µg/mg trichloroacetic acid, tetradecyl ester, 1.559µg/mg trichloroacetic acid, hexadecyl ester, 7.752µg/mg 2-octyne, 24.474µg/mg 4-methyl-1-hepten-4-ol acetate, 10.949µg/mg 1,4-octadiene, 31.879µg/mg 1-methyl -1,2,3,6-tetrahydropyridin and 16.169µg/mg 3-octyne. In *M. azedarach* leaves extract identified only one compounds with 34.116 retention time. The identified compound was 100.00µg/mg 11-cyclohexyldimethylsilyloxyundec-1-ene; while in *M. azedarach* bark extract with 30.740 - 35.379 retention time including; 0.361µg/mg 3-cyclohexene-1-ethanol, alpha. -ethenyl -. alpha., 3-dimethyl-6-(1-methylethylidene)-, 0.285µg/mg cyclopropane,1,1'-ethenylidenebis, 7.346µg/mg 1,4-cyclotadiene, 90.181µg/mg 6-octadecnoic acid, methyl ester, (*Z*)- and 1.827µg/mg *N*- (p-methoxyphenyl) -*N*' – (1,1-dicyanoethyl)-diazene. In case of *E. hirta* whole plant extract revealed twenty-eight compounds with 6.411 - 36.933 retention time. The identified compounds were mentioned in Table 3. (Figure 9 a, b, c, d, e).

Discussion

In the search for bioactive secondary metabolites from natural sources, ethnobotanical investigations and phytochemical screening remain the mainstays. However, computational techniques in preclinical drug discovery and development research play a significant role. (Ramya et al., 2022). Antioxidant phytochemicals are widely identified as being beneficial to disease prevention and human health, such as age-related degenerative brain disorders, cancer, coronary disease and infectious diseases (Corcoran et al., 2012).

In present work the percentage concentration yield of all plant parts was measured as a proportion of proficiency of the dissolvable utilized during extraction ad fractionation. Ethanol concentrates of *A. nilotica* whole branch and bark indicated % yield i.e., 16.8 and 24.0; *M. azedarach* leaves and bark indicated % yield i.e., 30.8 and 18.8; *E. hirta* whole plant indicated % yield i.e., 22.0. The overall trend of % yield of ethanol extract was *M. azedarach* > *A. nilotica* >*E. hirta*. Alam et al. (2020) used ethanol crude extracts for liquid-liquid partitioning by using solvents in increasing polarity to separate the components. Medicinal plants include a large range of chemical compounds that differ in polarity and

chemical characteristics. Each extract yielded differently due to the varied polarity and composition of the solvent.

Presence of phytoconstituents were confirmed by thin layer chromatography. It is frequently utilized to offer the first distinctive fingerprints of plants (Samkumar et al., 2019). Saleh et al. in 2015 used TLC to validate the presence of phytoconstituents in *A. nilotica* leaves, seeing different spots for petroleum ether, methanol 70%, and water extract. The solvent systems best for petroleum ether extract were Benzene: Chloroform in 1:1; for methanol 70% extract, ethyl acetate: toluene: formic acid in 4:4:1 and for methanol 70% extract the best solvent system was ethyl acetate: butanol: water: formic acid in 10:10:4:2, whereas six spots were observed in water extract. Munir et al. (2017) in *M. azedarach* methanolic leaf extracts revealed three distinct colored spots which showed the presence of anthocyanidin-3-glycosides, anthocyanidin-3, 5-diglycosides, or isoquercetin and rutinosides. Samkumar et al. in 2019 using different solvent ratios, *n*-hexane and ethyl acetate in increasing order of 10:0 to 5:5, and reported the presence of terpenoids in crude extracts of *E. hirta* stem, leaves, roots, whole plant, and cell suspension culture.

Sadiq et al. (2015) reported the FT-IR study of *A. nilotica* leaves, pods, and bark extracts; NH_3+ in NH_4OH due to NH_3 deformation, N-O nitro compounds due to N-O symmetric stretch, C-O-C in aliphatic ethers, Si-O in silicates, and C=O in carboxylic acid and ketones group frequencies were present in leaf and pod extracts but absent in the bark. While NH_2 in NH_4OH, C-OH in secondary alcohols, and CO stretch were absent. As a consequence of their FTIR analysis, they were able to establish that the plant extracts included alkanes, carboxylic acids, ketones, amines, alcohols, phenols, secondary alcohols, alkynes, alkyl halides, aromatic compounds. FTIR analysis of *M. azedarach* showed a wide peak that reflects vibrations of the (-OH) hydroxyl group and (C=C) aromatic ring-containing alkene (Ashraf et al., 2020). Enathi et al. (2022) also described that *M. azedarach* showed the hydroxyl group peak at 3283 cm^{-1}. Further two peaks reflected the aliphatic C-H symmetric and asymmetric groups at about 2923cm^{-1} and 2910 cm^{-1} respectively. The O-H groups and the aromatic carbon to carbon vibration (C-C) of the phenyl group were also observed. Sanjit et al. (2020) in *E. hirta* found the existence of OH, CH, C-O, C=O, C=C, C-N, NO2, Ar-O, and R-O-C-Cl in the chloroform, butanol, and ethyl acetate extracts respectively.

According to Boly et al. in 2021, thirteen polyphenol molecules were found in the aqueous extract and fractions of *A. nilotica* by HPLC analysis; gallic acid had the highest content in the aqueous extract, ethyl acetate and butanol fraction than the gallic acid content of the ethanol extract of *A. nilotica var nilotica*. Ahmed and Rao (2013) also discovered by HPLC analysis that rutin, quercetin, and kaempferol in ethanol were found *M. azedarach* leaves extract. Rabet et al. in 2017 by using HPLC discovered two caffeic acid hexoside compounds in *M. azedarach* extract. Caffeic hexoside derivative I was found in both immature and mature leaves and fruits, with the greatest concentration found in mature pulps. While caffeic acid hexoside derivative II was ubiquitous in leaves and fruits, it accumulated preferentially in ripe pulps. According to Samkumar et al. (2019), triterpenoid and taraxerol can be seen in the HPLC chromatogram of *E. hirta* stem, entire plant, and cell suspension culture. The number of total flavonoids in the acetone, chloroform, ethyl acetate, methanol, and hydro-alcohol extracts of *E. hirta* was calculated by Rajasudha and Manikandan (2019).

According to Sadiq et al. (2015) and Ramirez et al. (2013) the *A. nilotica* leaves and pods revealed the presence of proteins, saponins, tannins, phenols, alkaloids, glycosides, flavonoids, and phenols by LC-ESI-IT-MS/MS study. The barks did not contain alkaloids or glycosides. Epicatechin, epicatechin-5-gallate, ethyl gallate, gallic acid, vitexin, l-arabinoside, and caffeic acid hexose were all identified in high concentrations in the leaves. Epicatechin-5-gallate, m-digallic acid, epicat-echin, digallocatechin-5-gallate, gallic acid, diallyl glucose, caffeic acid hexose, and kaempferol were all present in equal amounts in the pods. Martha et al. (2020) described that *M. azedarach* ethyl acetate fraction contained triterpenoid, toosendanin and its derivative, 12-hydroxyamoorastatin and its acetyl derivate, ortho-dihydroxy flavanols of quercetin glycosides, 1-cinnamoyl-3-hydroxy-11-methoxymeliacarpinin, salannin, meliarachin, and salannal. Samkumar et al. (2019) discovered Taraxerol in an LCMS study of an acetone *E. hirta* plant extract, and triterpenoids. Selva et al., 2012 research also revealed that ethanolic extracts of *E. hirta* stem and leaves have several secondary metabolites like tannins, saponins, glycosides, and steroids.

Sheema et al. (2014) used GCMS to identify the

bioactive compounds in the chloroform extract of *A. nilotica*, including; 2,4-dimethylbutylphenol, 3,5,7-tris (trimethylsiloxy), and 3,4-di(trimethylsiloxy)phenyl Linolenic acid, linole-4-H-1-pyran-4-one, stearic acid, palmitic acid, 2-methylresorcinol, and 1,3,4-eugenol, respectively. Rehman et al. (2022) also studied the content of polysaccharides, polyphenols, amino acids, steroids, and fatty acid esters in the methanolic extract of *A. nilotica*. According to Shilaluke and Moteetee (2022) Twenty-six chemicals, including two esters, three alkanes, two diterpenoids, two methyl esters, two non-metal compounds, two organosilicons, and two triterpenoids, were found in acetone extracts of *M. azedarach*. In another study, Ali et al. (2015) found 13 bioactive phytochemicals in the MeOH extract of *M. azedarach*, including diethyl ester, diethyl ester, propanedioic acid, butanedioic acid, diethyl ester, trichloromethane, 2-piperidimethanamine, butanedioic acid, 2-pyrrolidinyl-methylamine, triethyl citrate, hydroxyl-, 2,5-dimethylhexane-2,5-dihydroperoxide, dithiocarbamate, *s*-methyl-, *n*-(2-methyl-3-oxobutyl), *y*-sitosterol, ethyl 9,12,15-octadecatrienoate, 2-hydroxy-1-(hydroxymethyl)ethyl ester, hexadecanoic acid, octadecane and 3-ethyl-5-(2-ethylbutyl). Gautam et al. (2022) studied purified Extract of endophytic fungus *Nigrospora sphaerica* separated from a pantropical weed, *Euphorbia hirta* which displayed an appearance of 9-hexacosene, which was followed by 2,4-Di-tertbutylphenol, while 1-undecanol indicated the lowest% composition in GCMS analysis.

Conclusion

According to current study analysis, the richness of active metabolites in extracts is strongly associated with therapeutic potentials. These extracts contain natural substances such as alkaloids, terpenes, quinones, and polyphenols that have been demonstrate in future to effectively limit the development of infectious disease. The current study has established the medicinal importance of *A. nilotica, M. azedarach* and *E. hirta* through its phytochemical profiling, biological activities. Plants having medicinal exploration has consider as main source in the detection of natural bioactive substances that have capability of stopping disease pathogenesis and protozoal enzymes. The experimental data of studies suggest that the presence of active compound introduce the therapeutic use and also based on assumption that Cholistan desert medicinal plants are rich source(s) that confers activities like antioxidant, anti-inflammatory, antitumor, anti-hypertensive, antibacterial, antiviral and antiprotozoal activities and also helpful in treating different infectious diseases that can be controlled/treated through alternate medicine approach.

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Contribution of Authors

Malik S: Collected the data, performed experiments and wrote manuscript
Sial N: Conceived idea and designed the experiment, edited and approved the final manuscript
Shahzad MI: Conceived idea and designed the experiment, edited and approved the final manuscript
Anjum S: Assisted in performing TLC
Javid A : Assisted in GCMS analysis
Rivera G: Conceived idea and designed the experiment, edited and approved the final manuscript

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