Acta Scientiarum

http://www.periodicos.uem.br/ojs/
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v46i1.72215

Identification of bioactive constituents in indigenously grown *Calotropis gigantea* and *Moringa oleifera* fractions by HPLC

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ABSTRACT. Plants are the most ubiquitous sources of bioactive organic compounds on earth. Active metabolites such as phytochemicals from medicinal plants have been investigated for the development of cutting-edge and biodegradable potent drugs as an alternative to ineffective modern treatment. The medicinal plants *Calotropis gigantea* and *Moringa oleifera* contain biochemical constituents that may have therapeutic qualities. The bioactive components in *C. gigantea* and *M. oleifera* leaves were analyzed using HPLC. HPLC analysis of several leaf fractions unveils the presence of quercetin, gallic acid, coumaric acid, caffeic acid, chlorogenic acid, ferulic acid, cinnamic acid, sinapinic acid, and vanillic acid in both plants. Furthermore, phytochemical study of these plants revealed the presence of alkaloids, flavonoids, saponins, tannins, terpenoids, and glycosides in different solvents. The identified chemicals have reported antioxidant and antitumor properties. The current study's findings suggest that *C. gigantea* and *M. oleifera* could be used as a source for a credible antioxidant agent that might later be isolated and used as lead candidates for the fabrication of antioxidant drugs that help stop or limit free radical damage and counteract oxidative stress, thereby preventing a variety of chronic and degenerative diseases.

Keywords: acetic acid; aqueous chloroform; *Calotropis*; phytochemical; HPLC; *Moringa*.

Received on May 14, 2024
Accepted on September 4, 2024

Introduction

Herbal plants are highly valued for their wide range of therapeutic properties, making them a crucial component of our natural resources. The therapeutic effects of these plants are attributed to the existence of secondary metabolites, which play a significant role in their medicinal characteristics. The surge in international trade demand can be attributed to the efficacy, affordability, purported lack of adverse effects, and use as a substitute for allopathic treatments. Natural herbal remedies are readily accessible in nature. Herbal products are not intrinsically more secure than conventional medications, as they contain pharmacologically active compounds, some of which have been linked to significant side effects (Başaran Pasli, & Başaran, 2022). The cultivation of medicinal plants has been established in several countries due to their substantial benefits to healthcare.

Plant-based medicines and herbal therapies are regarded as healthful, pure, and safe due to their derivation from natural resources (van Wyk & Prinsloo, 2020). Plant-based medicines and herbal therapies are regarded as healthful, pure, and safe due to their derivation from natural resources (Chaachouay & Zidane, 2024). Herbal medicines have effectively treated diseases due to their longstanding customary. Numerous naturally occurring alkaloids and chemicals demonstrate combined benefits, including antiviral, antibacterial, anti-protozoal, and antioxidant properties (Chaughule & Barve, 2024).

The knowledge acquired through traditional medicine has facilitated the continued investigation of medicinal plants for the production of pharmaceutical products (Mushtaq, Abbasi, Uzair, & Abbasi, 2018). Over 85-90% of the global population is contingent on traditional medicine systems to address many different diseases (Wangchuk, 2018).

Calotropis gigantea, usually referred to as Aak, Arka, or big Indian milkweed, is a plant that holds significant herbal value. Various phytochemicals have been identified in different parts of *C. gigantea*, particularly in the leaves. These include gigantin, calcium oxalate, α and β-calotropeol, beta-amyrin, fatty

acids (both saturated and unsaturated), hydrocarbons, acetates, benzoates, a mixture of tetracyclic triterpene compounds, and giganteol (Kumar, Suresh, & Kalavathy, 2013). The plant has numerous potential uses including biogas production, as a substitute for petroleum goods, water purification, energy plantation, fibre production, feed, latex or rubber manufacturing, and as a substitute for paper. In order to comprehend their pharmacological activity, it is necessary to scientifically assess them at the biochemical level (Sachin, Rani, Amresh, & Sathyamurthy, 2018). Various components of plants possess significant therapeutic potential in treating a wide range of ailments such as asthma, cold, epilepsy, fever, indigestion, leprosy, piles, and dermatological conditions. These components also demonstrate anti-inflammatory, anthelmintic, anticancer, and antitumor properties, as evidenced in numerous polyherbal formulations (Sachin, Rani, Amresh, Rajadurai, & Sathyamurth, 2018).

Another botanical species, *Moringa oleifera*, frequently known as drumstick, is a highly farmed plant of the monogeneric family Moringaceae. It is indigenous to India, Pakistan, Bangladesh, and Afghanistan, and can be found across tropical regions (Pareek et al., 2023). It is widely distributed, particularly in tropical regions. The various components of this plant, such as the root, bark, gum, leaf, fruit (pods), flowers, seed, and seed oil, have been utilized in traditional medicine in South Asia for treating a range of ailments. These include inflammation, infectious illnesses, as well as disorders related to the cardiac, digestive tract, hematological, and hepatorenal systems (Singh, Gautam, Sourav, & Sharma, 2022). It was documented to have antibacterial (Kekuda et al., 2010), anti-inflammatory (Mahajan, Mali, & Mehta, 2007), anti-diabetic (Nandave, Ojha, Joshi, Kumari, & Arya, 2009), antioxidant (Sultana, Anwar, & Ashraf, 2009), and anticancer (Parvathy & Umamaheshwarim, 2007) capacities. In a span of a decade, several significant advancements in analytical techniques, such as HPLC, TLC, FTIR, GC-MS, enzymatic and ultrasonic extraction emerged as potent tools for identifying bioactive chemicals in plants (Sawant et al., 2023). Analyzing the chemical constituents of herbs using various analytical methods is crucial for verifying the identity of the plant and its specific chemical compounds, hence differentiating it from other plant species and phytochemicals.

Precision in both quantitative and qualitative estimation is a crucial and indispensable need to ensure consistent quality of natural source medications. The examination of phytochemicals involves measuring their quality, which entails performing initial tests and analysing the active compounds using cutting-edge analytical methods. HPLC, is a highly adaptable and reliable method commonly employed in the extraction of natural substances. It is a chromatographic technique that effectively separates a mixture of compounds. Frequently, natural products are extracted and evaluated in a biological experiment to thoroughly analyze and understand their qualities. Multiple authors have documented the application of HPLC for the analysis and measurement of secondary metabolites in plant extracts, with a particular focus on phenolic chemicals, steroids, flavonoids, and alkaloids (Boligon et al., 2012; Boligon et al., 2013; Barbosa Filho et al., 2014; Colpo et al., 2014). High-Performance Liquid Chromatography (HPLC) is a commonly utilised method for assessing phenolic chemicals in plants (Adil et al., 2024).

Bioactive compounds possess polyphenolics, flavonoids with reported biological activities including antimicrobial, anti-cancerous, anti-inflammatory, anti-hypertensive, anti-diabetic, therefore bioactive component profiling is of imperative importance. Currently, there are no established official guidelines (platform) for herbal preparations. Currently, it is highly challenging to precisely identify the presence of each component in a formulation. Hence, key objective is to establish specific characteristics that enable the identification of the complete presence of the bioactive constituents in herbal plants. Several chromatographic and spectrophotometric techniques, along with the assessment of physicochemical qualities, can be employed to establish a consistent pattern for detecting the presence of distinct substances (Sachan, Vishnoi, & Kumar, 2016). We planned this study to identify the bioactive components and phytochemicals in the extracts of *C. gigantean* and *M. oleifera* leaves using the HPLC method. This investigation may offer useful insights on the potential use of these plants in traditional medicine.

Material and methods

Sample collection and preparation

Mature plants of *C. gigantea* (Voucher No. 03/2002; *C. gigantea*; Cholistan) and *M. oleifera* (Voucher No. 101-1-16) were employed. *C. gigantea* and *M. oleifera* plants were recognized and authenticated by botanist also through comparison with samples preserved in the herbarium of the Department of Botany, University

of Central Punjab, Lahore, Pakistan. The fresh matured leaves of both plant species were thoroughly rinsed with distilled water multiple times and then with deionized distilled water to eliminate any particles or contaminants. The leaves were dried in shade for 15 days at room temperature (20-22°C). Phytochemical components were analyzed for both species. Fresh mature leaves were ground into a fine powder using liquid nitrogen. In order to obtain the crude extract of *C. gigantea* and *M. oleifera* leaves, we added 30 grams of finely grinded powder from each sample in 250 mL of six different fractions separately: acetic acid, chloroform, methanol, distilled water, n-hexane, and n-butanol. Each fraction was placed in a separate flask and labelled accordingly.

Flasks were shaken at 160 rpm in an orbital shaker for 10 days at ambient temperature with constant stirring to improve the separation and extraction of *M. oleifera* and *C. gigantea* leaf extract. The extracts were filtered through 8 layers of muslin cloth and centrifuged at 8,000 ×g for 15 minutes. After that, each filtrate portion of each sample was subjected to a rotary evaporator. The filtrate was concentrated using a rotary evaporator at 45 ºC and 120 rpm, yielding a semi-solid extract. Dried extracts were dissolved in different solvents and labelled and kept at -20. The prepared organic fractions were then evaluated for HPLC profiling and phytochemical analysis. The dried crude extract was dissolved in the 100 mL mobile phase. After passing through filter paper and a 0.45 mm membrane filter (Millipore), the extract was injected into the HPLC.

Phytochemical screening

The current study entailed the examination of phytochemicals through screening several solvent extracts in methanol, butanol, n-hexane, acetic acid, chloroform, and aqueous solutions. Chemicals employed were of analytical grades (Sigma Aldrich Ltd). Extracts of *C. gigantea* and *M. oleifera* were screened for the presences of major phytochemicals such as alkaloids, terpenoids, tannins, steroids, glycosides, and quinones using standard procedures as reported earlier (Harborne, 1973; Trease & Evans, 1989; Sofowora, 1993).

HPLC and settings

Gallic acid, benzoic acid, ascorbic acid, quercetin, used as standards were of analytical grade (Sigma Aldrich Ltd). Samples were analysed using an RP HPLC method. Shimadzu VP HPLC System (SpectraLab Scientific Inc, USA with LC-10AT, SCL-10A, FCV-10AL, GT-104 and SPD-10AV UV/Vis Detector and a loop injector with a loop size of 20 µl. The peak area was computed using CLASS VP software. RP HPLC analysis was performed under isocratic conditions in RP column (Phenomenex Jupiter 15 µm C18 300 Å, 250 x 21.20 mm LC Column, SpectraLab Scientific Inc, USA). Using a flow rate of 0.7 mL $min.^{-1}$, 10 µL from each extracts were dissolved in 1 mL of distilled H_2O and injected into the column to identify the phenolic compounds in samples with varying wavelengths between 254 and 385 nm (Tai, Thongklay, Meunprasertdee, Kornthattalim, & Kaaewmanee, 2018; Uthirasamy et al., 2021). The peak area and retention time of extract were compared to standard curves at different standard concentrations. The results were obtained by comparing the peak regions at a certain wavelength of the sample. The mobile phase utilized in the HPLC analysis was ACN:H_2O (50:50-95-5), which was eluted using gradient elution. The column employed was a 4.6 x 250 mm RP Shimpack at a temperature of 30 °C, with a flow rate of 0.7 mL $min.^{-1}$. The analysis ended after 60 minutes, and the detection wavelengths deployed were 254 and 385 nm.

Results and discussion

The phytochemical properties of the two medicinal plants examined are outlined in Table 1. The aqueous extract exhibited a higher concentration of secondary metabolites in both plants compared to all other extracts. In both medicinal plants, aqueous showed the highest amount of active metabolites (Table 1).

Our research showed that the *C. gigantea* extract's butanol and n-hexane had the fewest active phytochemicals. The phytochemical screening of extracts from *C. gigantea* leaves revealed that the majority of phytochemicals, such as alkaloids, saponins, tannins, terpenoids, and cardiac glycosides, are found in aqueous, acetic acid, chloroform, and methanol extracts. The findings of our experiments are corroborated by previous papers (Hassan et al., 2006; Oladimeji, Nia, & Essien, 2006; Verma, Satsangi, & Shrivastava, 2013; Kumar, Sharma, & Vasudeva, 2021) which provide evidence and demonstrate the therapeutic properties of *C. gigantea* as a medicinal plant. In the n-hexane extract, no saponins, flavonoids, terpenoids, or glycosides were found. This particular profile has been previously documented (Jucá et al., 2013).

Our study results revealed that *M. oleifera* possesses many types of phytochemicals, including alkaloids, flavonoids, saponins, terpenoids, tannins, glycosides, coumarins, and quinones (Table 1). The presence of

phytochemicals varied depending on the type of solvent used in the extraction process. Acetic acid and aqueous extracts of *M. oleifera* revealed most of phytochemicals. Aqueous, acetic acid extracts unveiled the maximum phytochemicals. Quinones and glycosides were absent in all except aqueous and acetic acid (Table 1), whereas terpenoids were absent in butanol and acetic acid extracts. The findings of our investigation have been reinforced by Kasolo, Bimenya, Ojok, Ochieng, and Ogwal-Okeng (2010) who reported the presence of catechol tannins, gallic tannins, steroids, triterpenoids, flavonoids, saponins, anthraquinones, and alkaloids in methanol and water (aqueous) extracts of *M. oleifera* leaves. The study results have been reinforced by the work of Adekanmi, Adekanmi, and Adekanmi (2020), which showed that butanol, methanol, and chloroform extracts of moringa leaves contain a number of phytochemicals, including saponins, flavonoids, terpenoids, cardiac glycosides, and alkaloids (Adekanmi, Adekanmi, & Adekanmi, 2020; Kshirsagar, 2021).

Table 1. Phytochemical analysis of different extracts of *Calotropis gigantea* and *Moringa oleifera*.

Phytochemical tests	Phytochemicals in *Calotropis gigantea*					
	Methanol	Aqueous	n-hexane	Butanol	Acetic acid	Chloroform
Alkaloids	+	+	+	+	+	+
Saponins	+	+	-	+	+	-
Tannins	+	+	+	+	+	+
Flavonoids	+	+	-	-	+	+
Terpenoids	+	+	-	-	+	+
Cardiac glycoside	+	+	-	-	+	-
Quinones	-	+	-	+	-	-
Phytochemical tests	Phytochemicals in *Moringa oleifera*					
	Methanol	Aqueous	n-hexane	Butanol	Acetic acid	Chloroform
Alkaloids	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	-
Terpenoids	-	+	+	-	-	+
Cardiac glycoside	-	+	-	+	+	+
Quinones	-	+	-	-	+	-

+ = Present, - = Absent.

The preliminary phytochemical investigation of *M. oleifera* displayed the presence of flavonoids, phenol, tannins, and glycosides in all extracts, suggesting that these compounds are the predominant secondary metabolites in *M. oleifera*. However, the presence of compounds is directly proportional to the polarity of the solvent and the specific part (tissue) of the plant being examined (Anwar, Latif, Ashraf, & Gilani, 2007). The studies examining the active phytochemicals of the *M. oleifera* plant were inconsistent and their findings were not compatible with each other. variation in season and agroclimatic locations of plants (Iqbal & Bhangerm, 2006), genetic variations, cultivation techniques, drying operations, and extraction methods all potentially account for this discrepancy (Aliyu et al., 2021). Additional factors including the selection and concentration of the solvent, the proportion of liquid to solid, the size of the plant material particles, the pH level, the temperature, and the time frame of the procedure, can greatly impact the effectiveness of solvent extraction (Cacace & Mazza, 2003).

HPLC has demonstrated to be an efficient technique for quantifying phytochemicals derived from natural sources. TLC and HPLC techniques are the benchmark in the qualitative determination of the fractional amount of substances. The HPLC analysis was employed to determine the particular polyphenolic composition of all fractions of *C. gigantea* and *M. oleifera*. The aqueous fraction contains phenolic compounds, which are listed in Table 2 and Table 3. These compounds are characterised by peaks that have distinct retention periods (RT min.).

There were seven phenolic compounds identified in the aqueous extract of both C. *gigantea* and *M. oleifera*. These compounds are quercetin (2.940 min. 3.187 min), chlorogenic acid (15.067 min. 16.953 min.), gallic acid (4.893 min. 4.033 min.), caffeic acid (12.820 min.), m-coumaric acid (20.013 min, 20.353 min), sinapinic acid (26.433 min. 26.567 min.) and ferulic acid (22.380 min. 22.710 min.) (Table 2, Table 3, Figure 1B, Figure 3B).

Another study (Khalid et al., 2023) demonstrated the presence of chlorogenic acid, ferulic acid, gallic acid, and p-coumaric acid in both aqueous and ethanol-based fractions. This investigation revealed the presence of coumaric acid and sinapic acid in extracts of *M. oleifera*, which aligns with the findings reported by

Muzammil et al. (2023). Gallic acid, ferulic acid, and p-coumaric acid were previously found in aerial portions of *C. gigantea* using HPLC (Khasawneh et al., 2011).

Table 2. HPLC analysis of different solvents (fractions) from *Calotropis gigantea* Leaves extracts.

Fractions/Compound name	Area (%)	Retention time (min.)	Concentration (ppm)
Methanol			
Quercetin	0.6	3.060	0.46
Gallic acid	1.9	4.193	0.97
Caffeic acid	16.7	12.940	10.94
Aqueous			
Quercetin	0.9	2.940	0.43
Gallic acid	0.7	4.893	0.52
Caffeic acid	4.3	12.820	4.02
Chlorogenic acid	2.7	15.067	4.35
m-coumaric acid	4.7	20.013	1.16
Ferulic acid	2.3	22.380	3.41
Sinapinic (sinapic) acid	10.6	26.433	2.81
n-hexane			
Quercetin	0.8	2.947	0.34
n-butanol			
Quercetin	8.2	2.940	0.43
Benzoic acid	0.8	14.857	0.71
Chlorogenic acid	1.9	15.913	1.31
Cinnamic acid	8.0	25.093	2.43
Acetic acid			
Quercetin	1.0	2.940	0.51
Chlorogenic acid	3.6	15.933	2.58
p-coumaric acid	8.9	17.880	1.05
Ferulic acid	13.8	22.107	9.04
Sinapinic (sinapic) acid	23.4	25.873	6.63
Chloroform			
Quercetin	0.8	2.940	0.51
Gallic acid	4.880	4.4	1.98
Chlorogenic acid	7.6	15.400	7.09
p-coumaric acid	1.8	7.800	0.27
m-coumaric acid	11.0	19.853	1.58
Cinnamic acid	17.8	25.187	7.49
Sinapinic (sinapic) acid	6.7	26.460	1.14

Table 3. HPLC analysis of methanol, aqueous, *n*-hexane, *n*-butanol, ethyl acetate and chloroform fraction from *Moringa oleifera*.

Compound name	Area (%)	Retention time (min)	Concentration (ppm)
Methanol			
Quercetin	1.5	2.900	20.6748
Gallic acid	11.0	11.0	104.4056
Vanillic acid	1.4	1.4	9.1357
Fenilic acid	5.1	17.140	96.8501
Aqueous			
Quercetin	3.4	3.187	186.136
Gallic acid	46.7	4.033	1732.3527
Vanillic acid	3.6	13.020	234.3042
Chlorogenic acid	2.3	16.953	21.3881
m-coumaric acid	1.6	20.353	29.9059
Ferulic acid			
Sinapinic (sinapic) acid	2.2	26.567	24.3271
n-hexane			
Quercetin	0.1	2.867	0.2394
Gallic acid	1.0	4.140	1.1832
Caffeic acid	0.3	12.980	0.4060
Vanillic acid	0.4	13.853	0.3551
p-coumaric acid	7.8	18.047	3.2155
Sinapic acid	10.8	26.500	4.4611
n-butanol			
Quercetin	0.4	2.893	2.1073

Gallic acid	20.0	4.127	80.6025
Vanillic acid	4.2	13.700	12.6723
Ferulic acid	4.3	22.333	11.9883
Acetic acid			
Quercetin	0.4	2.860	1.0844
Gallic acid	26.6	4.133	50.9608
Vanillic acid	165.551	13.720	4.13885
Benzoic acid	177.182	14.733	18.7812
Sinapinic (sinapic) acid	287.953	26.153	10.0783
Chloroform			
Quercetin	0.1	2.887	0.1337
Gallic acid	2.0	4.373	1.6081
Benzoic acid	2.7	14.427	6.3818
Chlorogenic acid	7.5	15.227	13.1206
Cinamic acid	14.5	24.600	11.320
Sinapinic (sinapic) acid	9.7	26.193	2.8553

Our investigation of n-hexane fraction bioactive chemicals indicated significant differences between species. *C. gigantea* leaves n-hexane fraction contained only quercetin (2.947 min) (Table 2, Figure 1C), while *M. oleifera* endured gallic acid (4.140 min), caffeic acid (12.980 min), vanillic acid (13.853 min), p-coumaric acid (18.047 min), and sinapinic acid (26.500 min) (Table 3, Figure 3C). Our n-hexane fraction results coincide with the qualitative phytochemical detection in both species (Table 1). *C. gigantea* acetic acid fraction (Table 2, Figure 2A) had a similar profile to its aqueous fraction (Table 2, Figure 1B), but gallic acid and caffeic acid were absent. *M. oleifera* acetic acid fraction (Table 3, Figure 4A) contained gallic acid (4.133 min.), benzoic acid (14.733), and vanillic acid (13.720 min.), which were not found in *C. gigantea*. The polarity of solvents, species differences, and extraction procedures could account for this discrepancy. The HPLC analysis revealed the presence of five identifiable compounds in the chloroform extract (*C. gigantea, M. oleifera*) viz., quercetin (2.940 min. 2.887 min.), chlorogenic acid (15.400 min. 15.227 min.), gallic acid (4.4 min. 4.373 min.), cinamic acid (25.187 min. 24.600 min.), and sinapinic acid (26.460 min. 26.193 min.) (Table 2, Figure 2B, Table 3, Figure 4B). The methanol extract of both plants included two particular known compounds: quercetin (3.060 min. 2.900 min.) and gallic acid (4.193 min. 11.0 min.) (Table 2, Table 3, Figure 1A, Figure 3A).

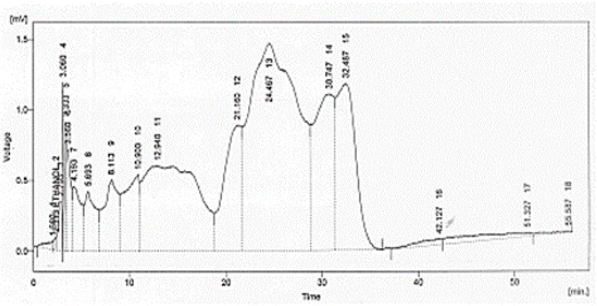
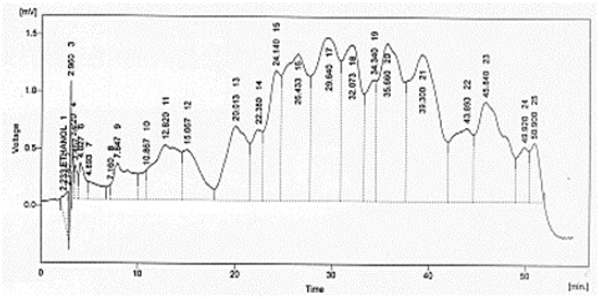


Figure.1A



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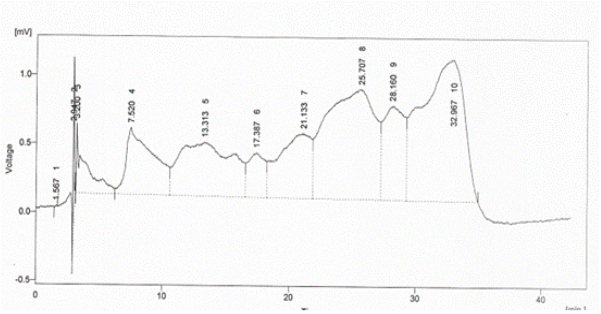

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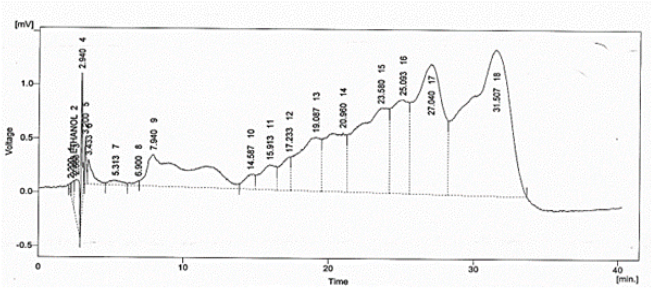

Figure.1D

Figure 1. (1A-1D). Figure 1A. HPLC analysis of methanol fraction from *Calotropis gigantea*, Figure 1B. HPLC analysis of aqueous fraction from *C. gigantea*, Figure 1C. HPLC analysis of n-Hexane fraction from *C. gigantea*, Figure 1D. HPLC analysis of n-butanol fraction from *C. gigantea*.

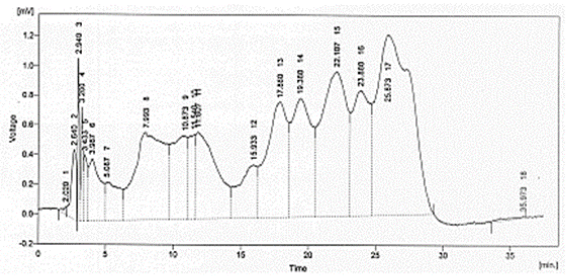

Figure.2A

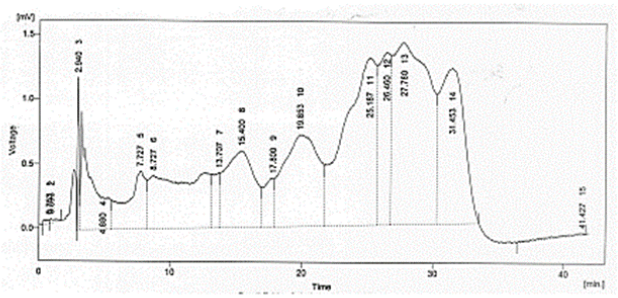

Figure.2B

Figura 2. (2A-2B) Figure 2A. HPLC analysis of acetic acid fraction from *Calotropis gigantea*, Figure 2B. HPLC analysis of chloroform fraction from *C. gigantea.*

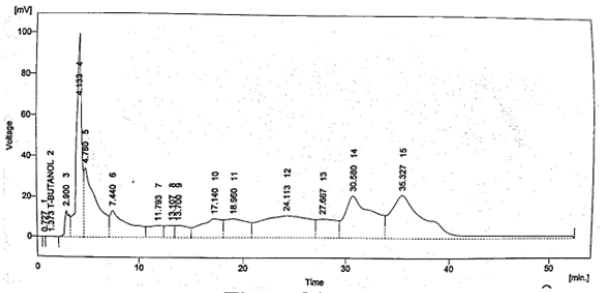

Figure.3A

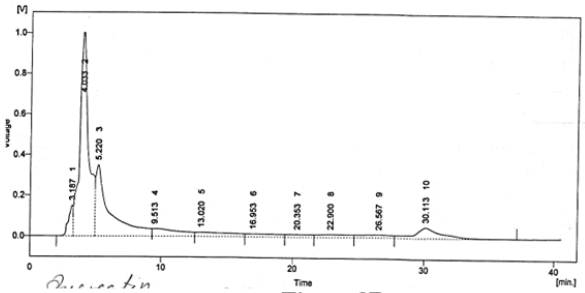

Figure.3B

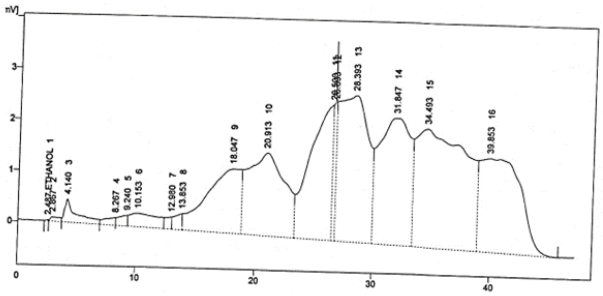

Figure.3C

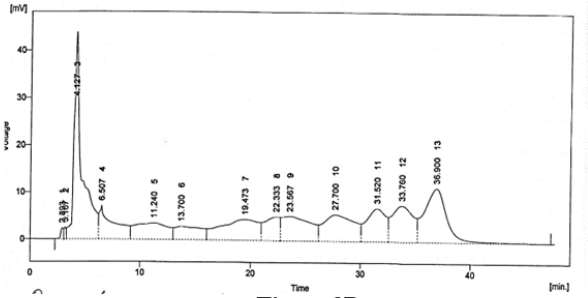

Figure.3D

Figure 3. (3A-3D). Figure 3A. HPLC analysis of methanol fraction from *Moringa oleifera*, Figure 3B. HPLC analysis of aqueous fraction from *M. oleifera*, Figure 3C. HPLC analysis of n-Hexane fraction from *M. oleifera*, Figure 3D. HPLC analysis of n-butanol fraction from *M. oleifera*.

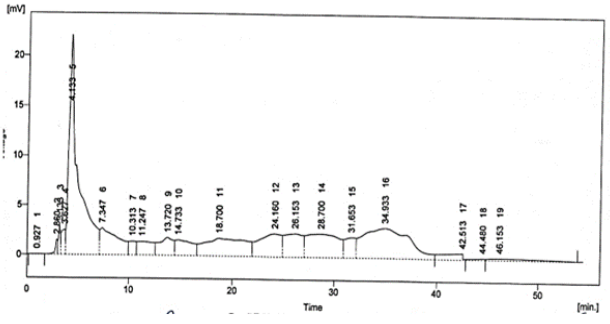

Figure.4A

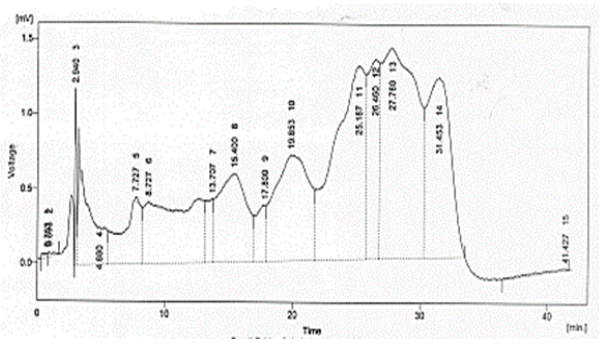

Figure.4B

Figure 4. (4A-4B) Fig 4A. HPLC analysis of acetic acid fraction from *Moringa oleifera*, Fig 4B. HPLC analysis of chloroform fraction from *M. oleifera.*

The plants exhibited distinct profiles of phenolic chemicals when exposed to two different solvents, n-hexane and n-butanol. The compounds quercetin (2.940 min.), benzoic acid (14.857 min), chlorogenic acid (15.913 min.), and cinamic acid (25.093 min.) were identified in the n-butanol extract of *C. gigantea* (Table 2, Figure 1D). Similarly, the n-butanol extract of *M. oleifera* encompassed quercetin (2.893 min.), gallic acid (4.127 min.), vanillic acid (13.700 min.), and ferulic acid (22.333 min.) (Table 3, Figure 3D). The phenolic

components detected in the methanol extract of *C. gigantea* leaves were identified using HPLC as gallic acid, quercetin, coumarin, cinnamic acid, and caffeic acid, as indicated in Table 2 and Figure 1A. These compounds have been previously explained (Sameeh & Mohamed, 2018). Therefore, these naturally occurring polyphenolic chemicals have the potential to serve as valuable antioxidants in several industries. Polyphenolics reported in our studies encompassing Quercetin has a wide range of applications in the food and nutraceutical industries (Srinivas, King, Howard, & Monrad, 2010), Chlorogenic acid used in food additives, food storage, food composition alteration, food packaging materials, functional food elements, and prebiotics (Wang et al., 2022), Gallic acid finds uses in the ink and dye industries, the food sector (antioxidants and preservatives), and most crucially, pharmaceutical industry (Goldberg & Rokem, 2009), Caffeic acid utilizes in the food sector, disease diagnosis, and environmental monitoring (Parasuraman et al., 2024), p-coumaric acid makes it appropriate for use in the pharmaceutical and food sectors (Kiliç & Yeşiloğlu., 2013), Ferulic acid has a broad application in the pharmaceutical, food, and cosmetics industries (Zduńska, Dana, Kolodziejczak, & Rotsztejn, 2018), Sinapic acid has a prospective application in food processing, cosmetics, and the pharmaceutical industry industry (Nićiforović & Abramovič, 2014), Benzoic acid esters have a widespread use as preservatives in the food, pharmaceutical, and cosmetic sectors (Vander Leek, 2024), Vanillic acid is a derivative of benzoic acid. It is frequently employed as a preservation agent, flavoring agent, and food additive in the food industry (Riaz ud Din et al., 2023). To use herbal medications, it is necessary to create a chemical profile utilising marker compounds. The plant metabolite matrix is exceedingly complex, and its composition may be easily analysed using biochemical approach like HPLC. The noticed polyphenolics using HPLC approach can be used to evaluate the quality of crude medications and a variety of herbal preparations containing these plants as a key ingredient (Table 4).

Our findings imply that *C. gigantea* and *M. oleifera* leaf extracts possess significant polyphenolics. These identified polyphenolics may be characterized for biological activities in future.

Table 4. Retention time of different bioactive compounds from *Calotropis gigantea* and *Moringa oleifera* based upon HPLC analysis.

RT (in min.) in different fractions)	Compound common name / IUPAC name	Molecular formula	Molecular weight
2.940, 2.947, 3.060	Quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one)	$C_{15}H_{10}O_7$	302.238 g mol^{-1}
4.4,4.193,4.893	Gallic acid (3,4,5-trihydroxybenzoic acid)	$C_6H_2(OH)_3CO_2H$	170.12 g mol^{-1}
7.800, 17.880	p-coumaric acid (trans-4-Hydroxycinnamic acid)	$C_9H_8O_3$	164.047 g mol^{-1}
12.820, 12.940	Caffeic acid (E)-3-(3,4-dihydroxyphenyl) prop-2-enoic acid)	$C_9H_8O_4$	180.16 g mol^{-1}
14.857	Benzoic acid (benzene carboxylic acid Carboxy benzene phenyl formic acid)	$C_7H_6O_2$	122.12 g mol^{-1}
15.067, 15.913, 15.933, 15.400	Chlorogenic acid (1S,3R,4R,5R)-3-[(E)-3-(3,4-dihydroxyphenyl) prop-2-enoyl] oxy-1,4,5-trihydroxycyclohexane-1-carboxylic acid)	$C_{16}H_{18}O_9$	354.31 g mol^{-1}
19.853, 20.013	m-coumaric acid (3-Hydroxycinnamic acid)	$C_9H_8O_3$	164.16 g mol^{-1}
22.107, 22.380	Ferulic acid (2E)-3-(4-hydroxy-3-methoxy phenyl) prop-2-enoic acid)	$C_{10}H_{10}O_4$	194.18 g mol^{-1}
25.187, 25.093	Cinnamic acid (2E)-3-Phenylprop-2-enoic acid	$C_9H_8O_2$	148.161 g mol^{-1}
25.873, 26.460, 26.433	Sinapinic acid (3,5-Dimethoxy-4-hydroxycinnamic acid)	$C_{11}H_{12}O_5$	224.21 g mol^{-1}
1.4, 3.6, 0.4, 165.551	Vanillic acid (4-hydroxy-3-methoxybenzoic acid)	$C_8H_8O_4$	168.14 g mol^{-1}

Conclusion

The study demonstrated that indigenous *C. gigantea* and *M. oleifera* are rich in active metabolites like alkaloids, flavonoids, tannins, and saponins. The HPLC method presented could be used to qualitatively and quantitatively analyse metabolites and bioactive chemicals in Apocynaceae and Moringaceae. The current investigation has documented the existence of phenolic compounds, including quercetin, chlorogenic acid, gallic acid, caffeic acid, ferulic acid, p/m-coumaric acid, cinnamic acid, sinapinic acid, benzoic acid, and vanillic acid, in the different fractions of *C. gigantea* and *M. oleifera.* The aqueous extract, being highly polar, has been determined to contain the majority of phenolic chemicals. The study indicated both *C. gigantea* and *M. oleifera* possess polyphenolics that might be powerful source of natural antioxidants. Additional comprehensive research is required to refine these phenolic components in order to utilise them as primary chemicals for the creation of innovative antioxidant medications. The current work could pave the way for additional biological and pharmacological research to isolate peculiar active compounds from these plant species in order to develop new medications to treat diseases. Future research should extract and characterise

individual phytochemicals, as well as evaluate them for biological activity. Although the pharmaceutical applications of *C. gigantea* and *M. oleifera* have received adequate attention, their general biological characteristics have not been thoroughly explored. In addition, the bioactivity relationship of *C. gigantea* and *M. oleifera*, which is imperative in establishing its therapeutic properties, has received little attention. Evaluating these fundamental aspects may improve their utilisation in the pharmaceutical industry and pave the door for innovative uses.

Acknowledgements

The authors would like to express their gratitude to Head, Biochemistry Department, University of Central Punjab, Lahore, Pakistan for their necessary assistance and support (resources) to carry out this research work successfully. We also appreciate the kind efforts and support of all the lab staff members for their timely help and support during this study.

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