J. Appl. Res. Plant Sci. Vol. 4(2), 653-661, 2023, https://doi.org/10.38211/joarps.2023.04.02.159

Research Article

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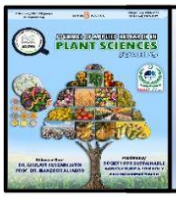

Available on https://www.joarps.org
Journal of Applied Research in Plant Sciences (JOARPS)
ISSN: 2708-3004 (Online), 2708-2997 (Print)

Identification and Quantification of Phenolic Compounds by HPLC-DAD and Antioxidant activity from Cordia Gharaf Plant

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Article Received 13-12-2022, Article Revised 20-03-2023, Article Accepted 15-05-2023.

Abstract

Numerous indigenous plants may grow on Pakistan's soil due to its suitable climate. A few of these are now exploited as a source of expensive pharmaceuticals with therapeutic benefits. Since 4000-5000 B.C., people have used these native herbs as medicines. The *Cordia gharaf* plant is one of them. There are several phytochemicals found in plants, including flavonoids, alkaloids, tannins, phenolic compounds, and others, that can protect against disease. Many phytochemicals have a broad range of biological activities that help to protect against chronic illnesses, and around the world, they have been used to treat several different human ailments. According to a World Health Organization (WHO) report, more than 80% of the world's population uses traditional medicines for treatment and maintaining their health, and many valuable medications have been extracted from these medicinal plants. Therefore, scientific investigation is necessary to make enormous profits from these priceless medicinal plants. Due to the importance of the primary and secondary metabolites produced by these medicinal plants, our purpose includes the idea of examining biologically active components from these plants. Keeping in mind the foregoing, the goal of our research is to examine the phytochemicals from the selected species of *Cordia gharaf* that have disease-preventive properties.

Keywords: *Cordia gharaf, phytochemical analysis, antioxidant, HPLC, UV-Visible*

Introduction

Medicinal plants have been in use for thousands of years for the treatment of different types of diseases and a large inventory of useful drugs has been isolated from them. The most significant and abundant sources of medicines are plants. Since the dawn of civilization, these herbs have been utilised all over the world to treat a variety of human illnesses (Amit Kumar *et al*., 2013; Sidhu *et al*., 2022). Ancient Egyptians have recommended chewing willow bark since the beginning of time to treat fever and headaches. Scientists eventually proved that the bark includes a salicylic acid component, which is an active ingredient utilized in the production of aspirin, many thousands of years after it was first discovered. At the moment, we are using substances that are sourced from plants (Parkash *et al*., 2005). Atropine has been utilized to treat some heart issues, and it's likely that a deadly nightshade plant extract has been used to relax eye muscles (Edoga *et al*., 2005). Phytochemicals, or chemical substances obtained from medicinal plants, have physiological effects on people. These bioactive phytochemical elements include amino acids, alkaloids, steroids, tannins, glycosides, saponins, phenolic acids, terpenoids, flavonoids, and carbohydrates, among others (Samejo *et al*., 20013). These components are produced by primary or secondary plant metabolism, the latter of which comprises a variety of substances used in medicine, science, agriculture, animal health, and some other sectors. (Max, R.A. *et al*., 2007: Vasu, K. *et al*., 2009).These phytochemicals, which come from several classes of substances, have been shown to have antimicrobial properties for all sorts

of microorganisms in vitro and may shield animals and people against several diseases, such as cancer, diabetes, and cardiovascular problems (Muthee, J. *et al*., 2016). Various phytochemicals exhibit therapeutic properties that help prevent chronic illnesses (Tiwari *et al*., 2016; Mangi *et al*., 2021). For instance, flavonoids have antimicrobial, antifungal, antidiarrheal, anti-inflammatory, and antibacterial properties, while tannins and alkaloids have antimicrobial, antidiarrheal, and anthelmintic properties; polyphenols have similar properties; coumarins have antiviral properties; terpenoids have antidiarrheal properties; polypeptides have antimicrobial properties; and lectins have antiviral properties (Siddiqui, S, *et al*., 2009; Krishniaah D, *et al*., 2009; Tiwari, P. *et al*., 2011). There are 300 species of *Cordia gharaf*, most of which are found in the tropics and subtropics of the world. It has 60 different species and grows to a height of 1 to 18 metres. More than 300 species of invertebrates as well as camels and cattle are consumed by the genus Cordia (Khan, S. *et al*., 2004). Despite being the size of a little tree, *Cordia gharaf* has a deep taproot that can reach 30 metres or more below the surface of the earth. The tree is native to Iran, Bhutan, India, Bangladesh, Afghanistan, Nepal, and Pakistan. It is widely distributed in Pakistan's Khyber PakhtunKhwan (KPK) and Sindh provinces (Malairajan P. *et al*., 2006). Phytomedicines, often known as plant-based medications, have been utilised for a very long time. Several plant parts, including the leaves, bark, flowers, roots, seeds, and fruits, can be used to obtain this (Mojab, F. *et al*., 2003). Due to the significance of this knowledge for the production of biochemical components, the study of these plants' chemical composition is extremely important (Parekh, J. *et al*., 2007). Additionally, this plant is used as a diuretic, and carminative, and to treat liver infections and splenic or inflammatory conditions. It is also used as an astringent for conditions including vaginal discharge. (Parekh, J. *et al*., 2008; Khan, S. *et al*., 2013).As a result of these plants' abundance of essential oils and secondary metabolites with therapeutic value, our goal includes the idea of researching physiologically active components from them.

Material and Methods

Plant material and Chemicals: The different *Cordia gharaf* plant parts (seed, stems, and leaves) were collected from Tandojam, Hyderabad Sindh, Pakistan in August 2020. All phenolic standards used were of the highest purity and obtained from Merck (Darmstadt, Germany).

Extraction of Free Phenolic Acids and Bound Phenolic Acids: Free phenolic and bound phenolic acids were determined by a reported method with slight variations (Memon*et al*., 2012).

Total Phenolic Content: The total phenolic content (TPC) of *Cordia gharaf* extracts was determined using the reported method Folin–Ciocalteu (FC) reagent (Memon*et al*., 2012; Boakye *et al*., 2015).

Total Tannin and Total Flavonoid Contents: Total tannin contents (TTC) and Total flavonoid contents (TFC) were determined by a reported method with slight variations (Siddiqui*et al*., 2017; Laghari*et al*., 2011; Criagg, G.M *et al*., 2001).

Radical scavenging activity (RSA): Free radical scavenging activities from different parts of *Cordia gharaf* extracts were determined by using a standard procedure (Amron and Konsue, 2018).

Statistical analysis: The results were evaluated using Microsoft Excel 2013 as mean±standard deviation. Minitab Software (version 16.1.1) was operated for data analysis.

Results

Phenolic acids profiling of various parts of *Cordia Gharaf* by HPLC-DAD: At a flow rate of 1 mL/minute, standards for 20 different phenolic acids were added to a chromatographic column (Table 1), (Memon *et al*., 2012). Characteristically, plant materials contain either free or conjugated versions of all phenolic chemicals. Simply free phenolic acids can be extracted and identified using a variety of solvents, such as ethyl acetate, methanol, aqueous acetone, etc, without the use of any acid or base. Conjugated phenolic acids can also be extracted using the hydrolysis of various plant extracts by an acid or base. Numerous thousands of phenolic compounds have been documented in the literature (Luthria, D. *et al*., 2006; Madhujitha, T., & Shahidi, F. 2009), employing acidic, basic, and enzymatic hydrolysis techniques or processes, the majority of which were extracted from various dry plant materials and identified as free phenolic acids. To determine the total amount of soluble-bound, free, and insoluble phenolic acids present in *Cordia gharaf* extracts, dried plant material was base hydrolyzed in the current investigation.

Table 1. Separation and identification of phenolic standards.

Standards	$t_{R\ (min)}$	R^2	Regression equation	λ_{max} (nm)
Gallic acid	7.89	0.999	y=305726x-249684	227, 272
2,4,6-THBA	9.51	0.998	y = 49119x+29082	216, 255, 292
Protocatechuic acid	13.16	0.997	y=530511x+112990	228, 259,294
Pyrogallol aldehyde	14.18	0.999	y=337860x+147020	234, 291
Protocatechuic aldehyde	14.35	0.998	y=548015x+303632	234, 281
Gentisic acid	14.92	0.999	y= 13444x-1829.4	232, 327
Sinapic acid	16.26	0.991	y=643555x–1E+06	255,294
β-resorcinolic acid	18.99	0.998	y=200138x+46398	255, 294
Hypogallic acid	19.61	0.998	y = 82657x - 14787	232, 314
vanilline	20.13	0.999	Y=626260x-138097	233, 281, 307
Vanillic acid	25.33	0.999	y= 289390x-82077	223, 260, 294
Caffeic acid	28.32	0.995	y=169059x-140031	233, 323
Chlorogenic acid	29.34	0.998	y = 97008x-33773	217,233, 327
Syringic acid	32.22	0.999	y=214749x-72422	225, 275
PHBA	35.18	0.999	y=88856x-14995	234, 308
p-coumaric acid	40.32	0.995	y=213962x-333316	232, 309
Ferullic acid	46.77	0.998	y=174006x+127640	235, 322
m-coumaric acid	47.59	0.999	y=533000x+78590	216, 232, 278
o-coumaric acid	48.75	0.999	y=7E+06x-2E+06	232, 277, 330
Cinnamic acid	49.05	0.992	y=568487x+305505	230, 280, 330

In the HPLC-DAD study of *Cordia gharaf* extracts, the leaves contain seven bound phenolic acids and three free phenolic acids (figs. 1 and 2), while the stem (figs. 3 and 4) and seed (fig. 5 and 6) both contain eleven bound phenolic acids where zero free phenolic acids are present in the stem and one free phenolic acid are present in seed correspondingly. In contrast, free phenolic acids were found to be more abundant in leaves (15.98 mg.g-1) than in stems (ND) and seeds (5.33 mg.g-1), which were discovered to have lower amounts of bound phenolic acids (34.43 mg.g-1) than stems (50.42 mg.g-1) and seeds (53.23 mg.g-1). As a result, the lowest amounts of protocatechuic acid (0.92 mg.g-1) and caffeic acid (1.56 mg.g-1) as found in the stems and seed, respectively. The amount of catechin acid was larger in the extracts of the leaves (11.92 mg.g-1), stems (14.86 mg.g-1), and seeds (15.37 mg.g-1), respectively. The findings are in good agreement with the literature (Robbins, R. J. 2003). Table 2 displayed the amounts of bound and free phenolic acids present in *Cordia gharaf* extracts from the leaves, seeds, and stems.

Total tannins, Total phenolic content, total flavonoids, and DPPH radical scavenging activity: Table 3 lists the number of total flavonoids, total phenolics, total tannins, and radical scavenging capacity in *Cordia gharaf* stem, leave, and seed extracts. The results demonstrated that total flavonoid and total tannin levels were found to be higher in leaves than in stems and seeds, whereas total phenolic content and antiradical activity were found to be higher in seed extracts. Total phenolic content was found to be between 31.78-58.00 mg.g-1, total flavonoids to be in the range of 21.00-33.78 mg.g-1, and total tannins to be in the range of 20.00-40.78 mg.g-1, whereas radical scavenging activity was determined to be between 55.01-120.23 μmol.100g^{-1} for *Cordia gharaf* leaf, stem, and seed extracts. Although total flavonoid and total tannin content were found to be higher in leaves compared to stems and seeds, the seed extracts also have a higher total phenolic content and significant antiradical activity. These results are in good agreement with the reported literature (Rubens F.V.D.S & Wagner F.D.G., 2004; Hostettmann K., *et al*., 2000).

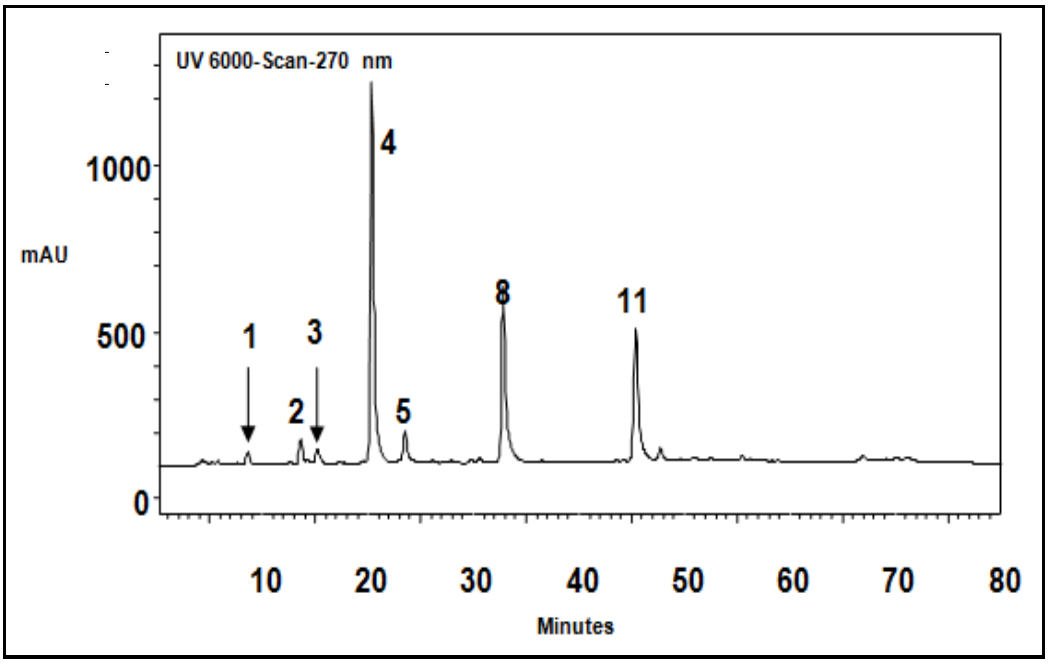

Figure.1: HPLC chromatogram of *Cordia gharaf* leaves BPA

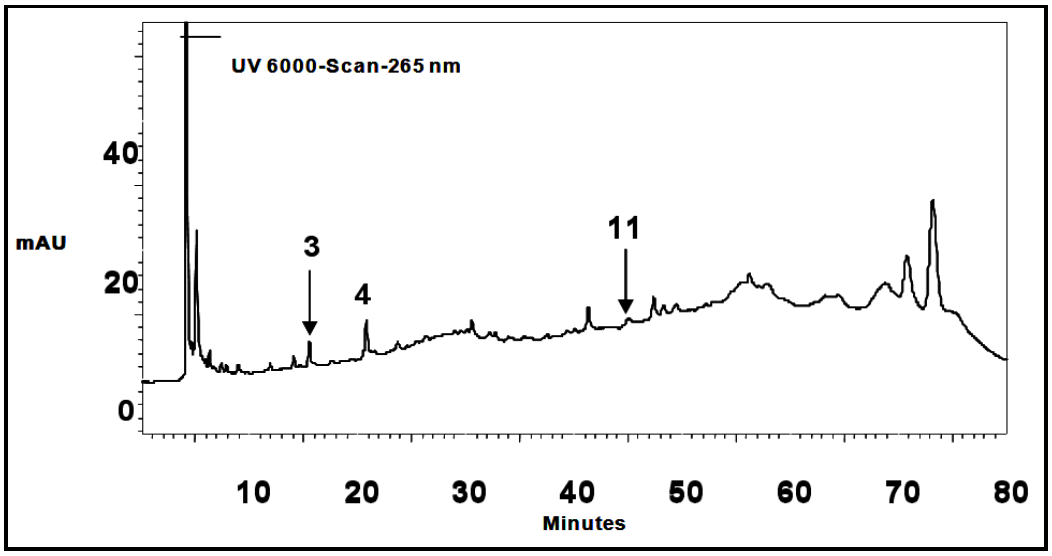

Figure. 2: HPLC chromatogram of *Cordia gharaf* leaves FPA

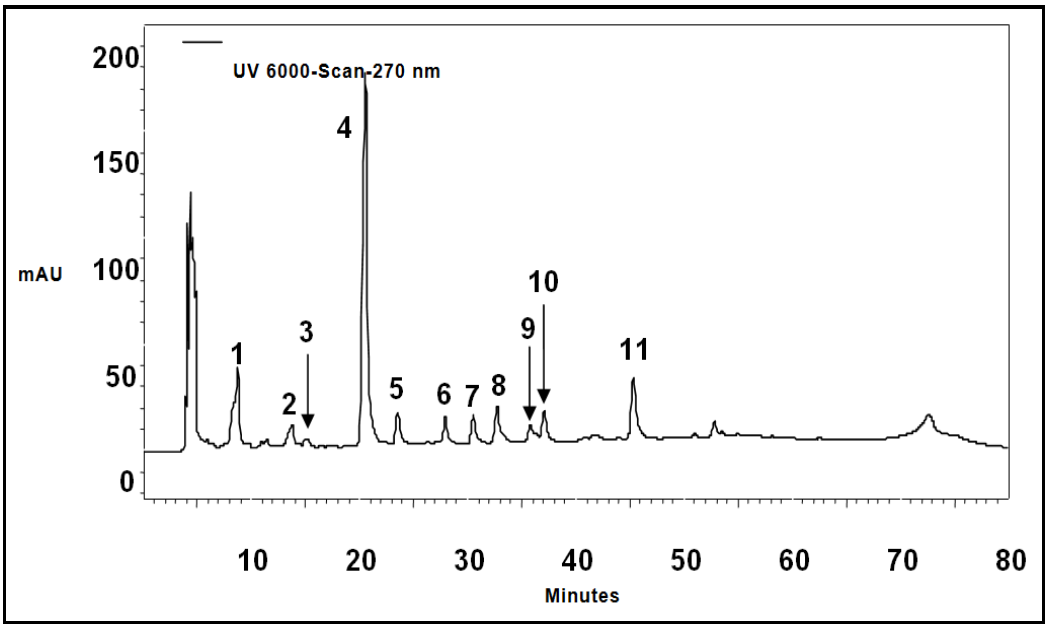

Figure. 3: HPLC chromatogram of *Cordia gharaf* stems BPA

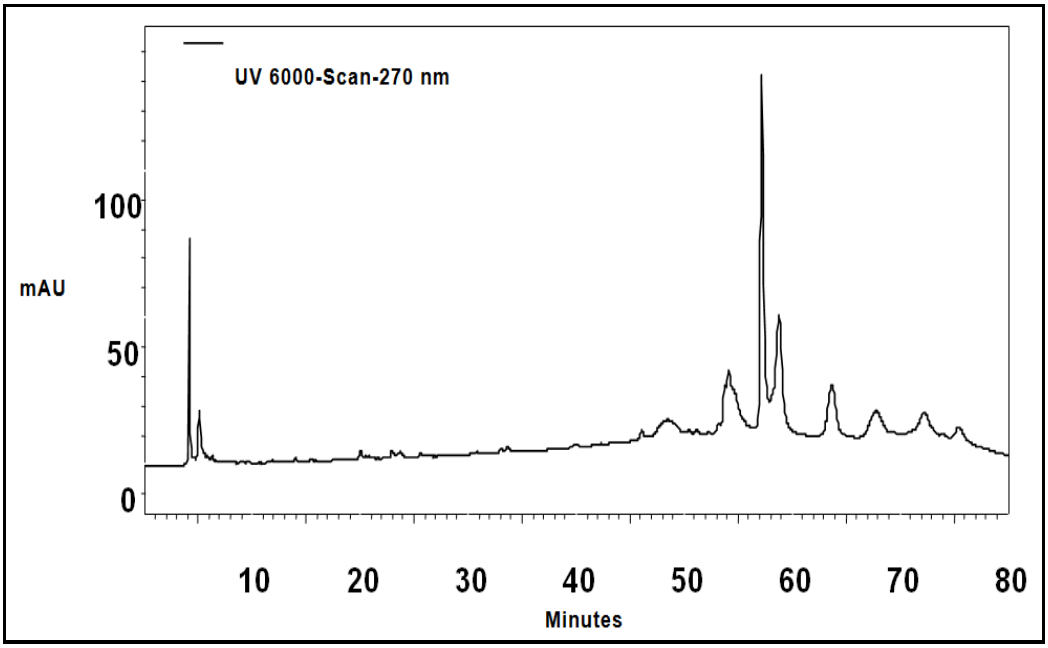

Figure. 4: HPLC chromatogram of *Cordia gharaf* stems FPA

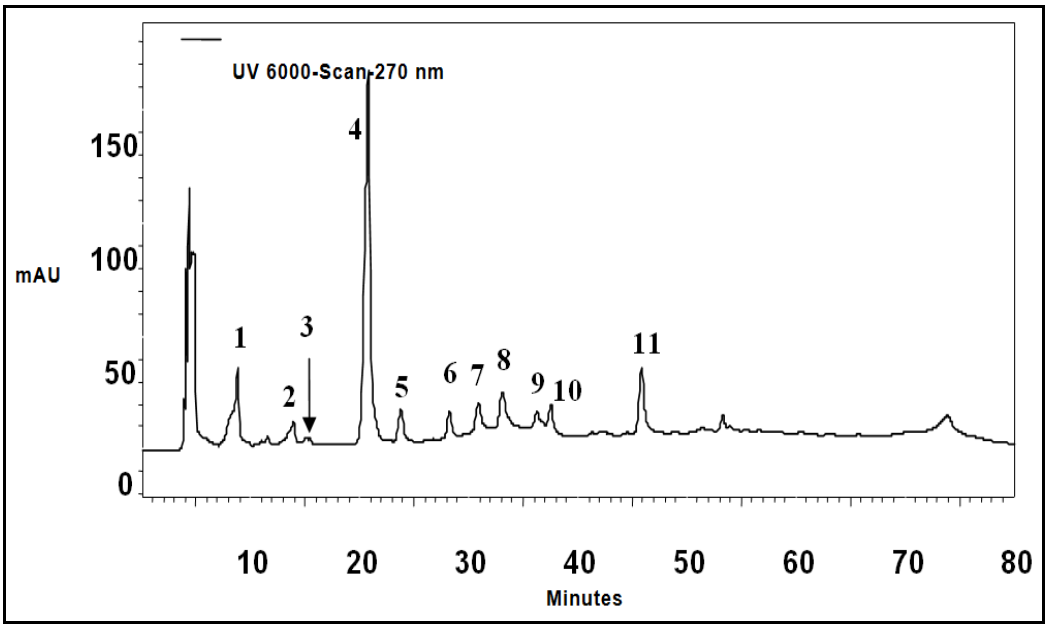

Figure. 5: HPLC chromatogram of *Cordia gharaf* seeds BPA.

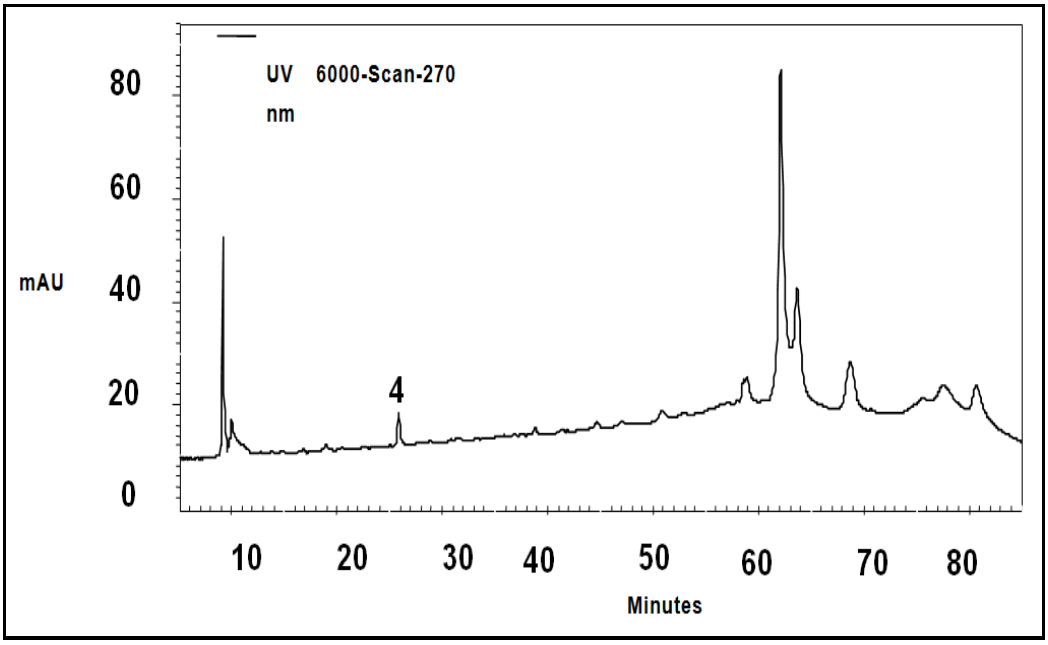

Figure. 6: HPLC chromatogram of *Cordia gharaf* seeds FPA

Table 2. free phenolic acids and bound phenolic acids in extracts (leaves, stems and seeds) of *Cordia gharaf*

S. No	Phenolic compounds	R.T(min)	Leaves mg.g–1		Stems mg.g–1		Seed mg.g–1	
			BPA	FPA	BPA	FPA	BPA	FPA
1	Gallic acid	8.89	3.36	ND	9.47	ND	6.87	ND
2	Unknown 1	13.91	2.27	ND	3.00	ND	2.40	ND
3	Protocatechuic acid	15.16	7.08	5.43	0.92	ND	1.44	ND
4	Catechin acid	20.88	11.92	6.22	14.86	ND	15.37	5.33
5	Sinapic acid	23.78	2.58	ND	2.55	ND	2.68	ND
6	Unknown 2	28.23	ND	ND	1.77	ND	4.11	ND
7	Vanillic acid	30.90	ND	ND	4.19	ND	3.88	ND
8	Caffeic acid	33.13	1.56	ND	1.04	ND	1.88	ND
9	Unknown 3	36.23	ND	ND	3.22	ND	2.77	ND
10	Unknown 4	37.51	ND	ND	2.32	ND	2.50	ND
11	*p*-coumaric acid	45.81	5.66	4.33	7.08	ND	9.33	ND
Total		**55**	**34.43**	**15.98**	**50.42**	**-**	**53.23**	**5.33**

Table 3. total phenolic, total flavonoid, total tannin, total phenolic content and radical scavenging activity in extracts of *Cordia gharaf* (leaves, stems and seeds)

S. No	Sample	TPC as Gallic Acid eq. (mg.g^{-1}±RSD)	TFC as rutin eq. (mg.g^{-1}±RSD)	TTC as catechin hydrate eq. (mg.g^{-1}±RSD)	TPA (mg.g–1± RSD)	Radical scavenging activity as Quercetin eq. (µmol.100g^{-1}±RSD)
1	Leave	31.78±0.22	33.78±0.48	40.78±1.38	34.43±1.73	55.01±2.41
2	Stem	50.77±1.13	27.77±0.16	23.77±0.26	50.42±2.26	85.74±2.37
3	Seed	58.00±2.24	21.00±0.33	20.00±0.13	53.23±2.87	120.23±3.16

Discussion

Proanthocyanidins, flavonols, and flavonol glycosides are extractable from plants using methanol and aqueous acetone, respectively. Flavonols and flavonol glycosides are also extractable from plants using water. Gallic acid esters, however, are not extractable due to their extremely high molecular weight (Ramya *et al*. 2011; Li, Y. *et al*., 2006). The phenolic acids, total phenolic, total flavonoids, total tannins, and antioxidant activity of the methanol extract of *Cordia gharaf* stems, leaves, and seeds were determined (Contini, M. *et al*., 2008). Methanol was selected as the extraction solvent for phenolic compounds due to the possibility of extracting other chemicals. Because many phenolic compounds are isomerized in sunlight (trans-cis conversion), react with oxygen in basic solution (quinone production), and react with methanol at normal temperature and pH, caution should be taken when extracting them (Singh *et al*., 2014). *Cordia gharaf* is found arch source of many important phenolic compounds which possess medicinal properties and can be used as traditional medicines because this is a local plant of Sindh which is available in every region and it can be the cheapest source of many phenolic compounds. Moreover, these important compounds are extracted from the plant and can be used in the pharmaceutical and cosmetic industry as they can be less costly phenolic compounds.

Conclusion

It is concluded that the amount of bound phenolic acids in leaves was lower 34.43 mg.g–1 than in seeds 53.23 mg.g–1 and stems 50.42 mg.g–1, while the amount of free phenolic acids was higher in leaves at 15.98 mg.g–1 and lower in seeds 5.33 mg.g–1. Total phenolic content was found to range from 31.78 to 58.00 mg.g–1, total flavonoids from 21.00 to 33.78 mg.g–1, total tannins from 20.00 to 40.78 mg.g–1, and total DPPH radical activity from 55.01 to 120.23 µmol.100g-1 for the *Cordia gharaf* leaves, stems, and seeds, respectively. The results showed that total phenolic content and antiradical activity in seed extracts are higher than in stem and seed extracts, whereas total flavonoid content and total tannin content were found to be higher in leaves than in stem and seed extracts. In conclusion, *Cordia ghraf* plant can be consider as a potential source of natural antioxidant due to the presence of various phenolic compounds. The HPLC-DAD method used in this study can be useful for the identification and quantification of phenolic compounds in other plants. Further studies are needed to explore the potential application of *Cordia ghraf* plant as a natural antioxidant in the food and pharmaceutical industries.

Acknowledgement

We are highly thankful to the Institute of Advanced Research Studies in Chemical Sciences, and Dr. M. A. Kazi Institute of Chemistry, University of Sindh, Jamshoro for instrumental facilities.

Conflict of Interest

The author declares that there is no conflict of interest.

References

Amron, N. A., & Konsue, N. (2018). Antioxidant capacity and nitrosation inhibition of cruciferous vegetable extracts. International Food Research Journal, **25**(1), 65-73.

Boakye, A. A., Wireko-Manu, F. D., Agbenorhevi, J. K., &Oduro, I. (2015). Antioxidant activity, total phenols and phytochemical constituents of four underutilised tropical fruits. International Food Research Journal, **22**(1).

Contini, M., Baccelloni, S., Massantini, R., &Anelli, G. (2008). Extraction of natural antioxidants from hazelnut (Corylusavellana L.) shell and skin wastes by long maceration at room temperature. Food Chemistry, **110**(3), 659-669.

Cragg, G. M., & Newman, D. J. (2001). Natural product drug discovery in the next millennium. Pharmaceutical biology, **39**(sup1), 8-17.

De Souza, R. F., & De Giovani, W. F. (2004). Antioxidant properties of complexes of flavonoids with metal ions. Redox Report, **9**(2), 97-104.

Edeoga, H. O., Okwu, D. E., & Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. African journal of biotechnology, **4**(7), 685-688.

Jigna, P., &Sumitra, C. (2008). Phytochemical screening of some plants from western region of India. Plant Archives, **8**(2), 657-662.

Khan, S., Khan, G. M., Mehsud, S., Rahman, A., & Khan, F. (2004). Antifungal activity of Tamarixdioica—an in vitro study. Gomal Journal of Medical Sciences, **2**(2).

Khan, S., Ullah, F., &Mahmood, T. (2013). In vitro antimicrobial and cytotoxic activity of TamarixdioicaRoxb.leaves. *Turkish Journal of Biology*, **37**(3), 329-335.

Krishnaiah, D., Devi, T., Bono, A., &Sarbatly, R. (2009). Studies on phytochemical constituents of six Malaysian medicinal plants. Journal of medicinal plants research, **3**(2), 067-072.

Kumar, A., Rahal, A., Chakraborty, S., Tiwari, R., Latheef, S. K., & Dhama, K. (2013).Ocimum sanctum (Tulsi): a miracle herb and boon to medical science-A Review. Int J Agron Plant Prod, **4**(7), 1580-9.

Kurt, H., Andrew, M., Karine, N., & Jean-Luc, W. (2000). The Potential of African Plants as a Source of Drugs. Current Organic Chemistry, **4**(10), 973-1010.

Laghari, A. Q., Memon, S., Nelofar, A., & Laghari, A. H. (2011). Extraction, identification and antioxidative properties of the flavonoid-rich fractions from leaves and flowers of Cassia angustifolia. American Journal of Analytical Chemistry, **2**(08), 871.

Li, Y., Guo, C., Yang, J., Wei, J., Xu, J., & Cheng, S. (2006). Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Food chemistry, **96**(2), 254-260.

Luthria, D. L., Mukhopadhyay, S., &Krizek, D. T. (2006). Content of total phenolics and phenolic acids in tomato (Lycopersiconesculentum Mill.) fruits as influenced by cultivar and solar UV radiation. Journal of food composition and analysis, **19**(8), 771-777.

Madhujitha, T., & Shahidi, F. (2009).Antioxidant potential of barley as affected by alkaline hydrolysis and release of insoluble-bound phenolics. Food Chemistry, **117**, 615–620.

Malairajan, P., Gopalakrishnan, G., Narasimhan, S., &Veni, K. J. K. (2006). Analgesic activity of some Indian medicinal plants. Journal of ethnopharmacology, **106**(3), 425-428.

Mangi, J., Jat, A., Soomro, N., Pirzada, A. J., &Sidhu, A. R. (2021). Assessment Of Phenolic Compounds, Antimicrobial Activity And Free Radical Scavenging Potency Of Three Selected Vegetables. Pakistan Journal of Agriculture, Agricultural Engineering and Veterinary Sciences, **37**(2), 97-104.

Max, R. A., Kimambo, A. E., Kassuku, A. A., Mtenga, L. A., & Buttery, P. J. (2007). Effect of tanniniferous browse meal on nematode faecal egg counts and internal parasite burdens in sheep and goats. South African Journal of Animal Science, **37**(2), 97-106.

Memon, A. A., Memon, N., Bhanger, M. I., &Luthria, D. L. (2013). Assay of phenolic compounds from

four species of ber (Ziziphusmauritiana L.) fruits: comparison of three base hydrolysis procedure for quantification of total phenolic acids. Food chemistry, 139(1-4), 496-502.

Memon, A. A., Memon, N., Luthria, D. L., Pitafi, A. A., &Bhanger, M. I. (2012). Phenolic compounds and seed oil composition of Ziziphusmauritiana L. fruit. Polish Journal of Food and Nutrition Sciences, **62**(1).

Mojab, F., Kamalinejad, M., Ghaderi, N., &Vahidipour, H. R. (2010). Phytochemical screening of some species of Iranian plants. Iranian Journal of Pharmaceutical Research, (2), 77-82.

Muthee, J. K., Gakuya, D. W., Mbaria, J. M., &Mulei, C. M. (2016). Phytochemical screening and cytotoxicity of selected plants used as anthelmintics in Loitoktok Sub-County, Kenya. J Phytopharmacol, **5**, 15-19.

Olusola, A., Olajide, O. O., Afolayan, M., & Arifalo, K. M. (2011). Preliminary phytochemical and antimicrobial screening of the leaf extractPilostigmareticulatum (dc) Hochst. African Journal of Pure and Applied Chemistry, **5**(3), 43-46.

Parekh, J., &Chanda, S. (2007). Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. African Journal of Biomedical Research, **10**(2).

Prakash, P. A. G. N., & Gupta, N. (2005). Therapeutic uses of Ocimum sanctum Linn (Tulsi) with a note on eugenol and its pharmacological actions: a short review. Indian journal of physiology and pharmacology, **49**(2), 125.

Premanath, R., Sudisha, J., Devi, N. L., &Aradhya, S. M. (2011). Antibacterial and anti-oxidant activities of fenugreek (Trigonellafoenumgraecum L.) leaves. Research Journal of Medicinal Plant, **5**(6), 695-705.

Robbins, R. J. (2003). Phenolic acids in foods: an overview of analyticalmethodology. Journal of Agriculture and Food Chemistry, **51**, 2866–2887.

Samejo, M. Q., Memon, S., Bhanger, M. I., & Khan, K. M. (2013). Essential oil constituents in fruit and stem of Calligonum polygonoides. Industrial crops and products, **45**, 293-295.

Siddiqui, M. S., Memon, A. A., Memon, S., &Baloch, S. G. (2017). Cuscutareflexa as a rich source of bioactive phenolic compounds. Journal of Herbs, Spices & Medicinal Plants, **23**(2), 157-168.

Siddiqui, S., Verma, A., Rather, A. A., Jabeen, F., & Meghvansi, M. K. (2009). Preliminary phytochemicals analysis of some important medicinal and aromatic plants. Advances in biological research, **3**(5-6), 188-195.

Sidhu, A. R., Basit, A., Hayat, A., Mangrio, S., Arain, S., Khalid, T., .& Elhakem, A. (2022). Quality characteristics, phytochemical analysis, and antioxidant of extract Cuscutareflexa (Roxb.). NotulaeBotanicaeHortiAgrobotanici Cluj-Napoca, **50**(3), 12691-12691.

Singh, P., Vishwakarma, S. P., & Singh, R. L. (2014). Antioxidant, oxidative DNA damage protective and antimicrobial activities of the plant Trigonella foenum-graecum. Journal of the Science of Food and Agriculture, **94(**12), 2497-2504.

Tiwari, B. K., Abidi, A. B., Rizvi, S. I., &Pandey, K. B. (2016). Phytochemical screening and evaluation of antioxidant potentials of some Indian medicinal plants and their composite extract. Ann. Phytomed, **5**(1), 99-103.

Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011). Phytochemical screening and extraction: a review. Internationale pharmaceutica sciencia, **1**(1), 98-106.

Ukuri, V., Jakku, V. G., &Aruri, S. (2009). Biomolecular and phytochemical analyses of three aquatic angiosperms. African journal of microbiology research, **3**(8), 418-421