https://doi.org/ 10.48047/AFJBS.6.5.2024. 10665-10680

African Journal of Biological Sciences

Antioxidant bioactivity and compound profiling through high performance liquid chromatography in *Stevia rebaudiana* and *Withania coagulans*

Jawad Ali[1], Asim Ali[1], Samiya Rehman[1], Saman Zulfiqar[2], Akram Naveed[3], Uzair Malik[4], Yasir Nawaz[5], Fouzia Tanvir[4], Aqeela Nawaz[5]

1 Department of Biochemistry, University of Okara, Pakistan

2 Department of Botany, The Govt. Sadiq College Women University Bahawalpur, Pakistan

3 Department of Chemistry, Government College University Faisalabad, Pakistan

4 Centre of Biotechnology and Microbiology, University of Peshawar, Pakistan

5 Department of Zoology, Faculty of Life Sciences, University of Okara, Okara, Pakistan

Corresponding author:

royyasirnawaz@gmail.com

Department of Zoology, Faculty of Life Sciences, University of Okara, Okara, Pakistan

Article History
Volume 6, Issue 5, 2024
Received: 22 May 2024
Accepted: 03 Jun 2024
doi: *10.48047/AFJBS.6.5.2024.10665-10680*

Abstract

Withania coagulans Dunal and Stevia rebaudiana are useful plants with a significant role in Ayurvedic medicine. The presence of biomedical compounds as secondary metabolites confers antimicrobial, anti-inflammatory, anti-oxidant, and anti-cancer activities to medicinal plants.

The total flavonoid and total phenolic content of Withania coagulans were determined using the aluminum chloride colorimetric and modified Folin-Ciocaltue methods respectively, while flavonoid content was measured by AlCl3 colorimetric method. *Withania coagulans* leaves obtained from Sargodha contained the greatest total phenolic content (47.33± 2.50 GAE/g DW). Mianwali leaf extract has highest flavonoid content QE/g DW (40.66 ±2.00 mg/g DW) and reducing power

32.662.5 Vit C equiv mg/g DW. *Stevia rebaudiana* leaves have total flavonoid content of 19.2±0.32, 22.9±0.18, and 13.79±0.17 mg Catechin/g in methanolic, ethanolic, and aqueous extracts, respectively. HPLC analysis of leaves of *Stevia rebaudina* revealed that rutin (0.14%), quercetin-3- D-glycoside (0.05%), and luteolin (0.03%) were most abundant phytochemicals in samples. Catechins were quantified at 280nm and included gallic acid (0.01%), epigallocatechin (0.72%), epicatechin (0.07%), gallocatechin (0.09%), and epicatechin gallate. To conclude phenolic content was highest in Sargodha leaves. Mianwali leaves extract had the greatest concentration of flavonoid. The total flavonoid content for the stevia rebaudiana plant was found in good concentration in all extracts.

Keywords: Antioxidants, HPLC, total flavonoid content, total phenolic content, reducing power

Introduction

Plant-based medicines are used to treat various ailments worldwide. Most of these medicinal plants grow naturally in the wild. Wild medicinal plants are important for both local medicinal purposes and global trade (Sureshkumar, Silambarasan et al. 2017). The knowledge of using these plants as medicine was acquired from local people by different trials and errors, and this information has been approved down through generations. Plant-based medicines are considered more human and environmentally friendly due to their fewer adverse effects (Sisubalan, Velmurugan et al. 2014). In Pakistan and other under developed nations where agriculture is main livelihood, plants serve as more than just a balance to the ecosystem; they are also sources of fuel, medicine, food, and animal feed (Azhar, Aris et al. 2015). The presence of beneficial phytochemicals and antioxidants makes these plants potent therapeutic agents. Medicinal plants are important for human life because they are a source of antioxidants (Chanda and Dave 2009, Pammi, Suresh et al. 2023) acting as drugs used as therapeutic traditional and modern medicine (Hosseinzadeh, Jafarikukhdan et al. 2015, Süntar 2020). Medicinal plants contain compounds that have therapeutic effect or they can act as precursors for synthesis of other therapeutically potent molecules (Sofowora, Ogunbodede et al. 2013).

Antioxidants are naturally occurring compounds prevent the disease by inhibiting the damage caused by cellular oxidation (Admassu and Kebede 2019). Antioxidants are mechanistically active in the various processes of life including differentiation of cells, neutralization of free radicals, changing the metabolism of endogenous hormones, enhancing enzymatic activity leading to apoptosis of cancer cells, DNA replication and its repair process (Hacışevki and Baba 2018). Antioxidants are defenders of the body against reactive oxygen species, decreasing the risk of chronic diseases (Stanner, Hughes et al. 2004).

Withania coagulans seeds have diuretic, anti-inflammation and ophthalmic-treating capacities, while buds of flower have anthelmintic characters (Maurya, Kalita et al. 2010, Mudassir, Hussain et al. 2018, Manjuladevi, Shilpa et al. 2019). The dry fruits of *Withania coagulans* anti-bacterial (Gurunathan, Qasim et al. 2020), anti- diabetic (Ram, Sardar et al. 2021) and anti-microbial properties (Peerzade, Mutturi et al. 2024)

while ripe fruits are used for the wound healing (Melguizo-Rodríguez, Illescas-Montes et al. 2021). Stevia rebaudiana an important in-vitro grown medicinal plant is used in the food industry as a low-calorie sweetener as well as a therapeutic plant (Gupta, Purwar et al. 2013, Amin, Ozgen et al. 2017). Stevia plants have excessive amount of micro as well as macro minerals and possesses medicinal properties likeantiviral (Jahangir Chughtai, Pasha et al. 2020), antidiabetic (Kurek and Krejpcio 2019) anticancer (Iatridis, Kougioumtzi et al. 2022) and antimicrobial properties (Ortiz-Viedma, Romero et al. 2017, Lemus-Mondaca, Vega-Gálvez et al. 2018). The purpose of this study was the chemical profiling of *Stevia rebaudiana* leaves and the antioxidant potential of medicinal plants including *Withania coagulans* and *Stevia rebaudiana.*

Materials and Methods

Sampling and handling of *Withania Coagulans*

W. coagulans leaves were obtained from three various sites in Pakistan. Plants were then divided into parts. Leaves were dried and crushed into powder (100 meshes), and samples were kept at 25°C under sterile conditions prior to extraction and assays.

Methanolic extraction of *W. coagulans*

A 250 mg sample was placed into pre-weighed Eppendorf tubes. Each sample was then mixed with 500 ml of a 1:1 chloroform and methanol solution. The samples were sonicated for 5 minutes, followed by 1 minute of vortexing. This sonication and vortexing cycle was repeated three times. Afterwards, the samples were centrifuged at maximum speed for 5 minutes. The green supernatant was then transferred into separate pre-weighed Eppendorf tubes (wi). The remaining debris was treated twice with the same procedure, with the supernatants added to the previous one. At room temperature, the Eppendorf's were allowed to dry. After that, the dried Eppendorfs were weighed once more (wf).

wf - wi = weight of the isolated extracts

The dried material was then dissolved in 100mg/ml DMSO, and the samples were sonicated for five minutes to remove any lumps. Samples were then subjected to antioxidant assays.

Total phenolic content assay of *W. coagulans*

By utilizing Modified Folin- Ciocaltue Method Total Phenolic Content of W. coagulans plant was evaluated (Wolfe, Wu et al. 2003).

Sample Preparation and Procedure of *W. coagulans*

About 100 ppm sample and control concentrations were used. The volume of Folin's reagent and sodium carbonate was maintained at 99 µl. There were 200 µl in the total reaction mixture. Gallic acid and DMSO were taken as positive and negative controls, respectively.

The test sample was incubated for 5 minutes after mixing with 99 µL Folin ciocalteu reagent (1:10) with distilled water. The reaction mixture was then given 99 µL of sodium carbonate (2000 g/L). For color development, the mixture was shaken rigorously for mixing, after that whole mixture was placed in the dark place for 90 minutes at 37º C. Then sample was utilized to measure the absorbance of sample at 760 nm, while DMSO was used as blank. Total phenolic content of samples was measured by:

$$Y = 0.0732X – 0.0205$$

Where 'Y' represents the sample's absorbance and 'X' represents the sample's total phenolic content in µg/ml.

Total flavonoid content assay of *W. coagulans*

Aluminum chloride (AlCl3) colorimetric technique was employed for determining the Flavonoids content of *W. coagulans* (Chang, Yang et al. 2002).

Sample Preparation and procedure of *W. coagulans extraction*

The 100ppm Sample solution was prepared. The reaction mixture's total volume was kept at 200 µL. The positive and negative controls were Quercetin and DMSO, respectively. AlCl3colorimetric technique was utilized for determining the Flavonoid Concentration. Each sample was mixed separately with 19 µl of 10% AlCl3, 19 µl of 1 M potassium acetate, and 200 µl of distilled water were mixed separately with each sample. The reaction mixture was maintained at 37 º C for 30 minutes, after which the absorbance was measured at 415 nm. The following formula was used to calculate the total flavonoid content of the sample:

$$Y = 0.0101X – 0.004$$

Here ‘Y' and ‘X' is Sample absorbance and the sample's total flavonoid content respectively. It is measured in µg/ml.

Reducing power assay of *W. coagulans*

Reducing power of sample was evaluated by the procedure of (Kumar and Pandey

2013).

Sample Preparation and procedure of *W. coagulans*

Samples solution of 100ppm concentrations was prepared. Using 0.2 M phosphate buffer, the total volume of the reaction mixture was enhanced to 200 µL. The remaining reagents' concentration was maintained at the same level. Positive and negative control, respectively, were ascorbic acid and DMSO.

Samples solution of 100ppm concentrations was prepared, and the concentration of 0.2 M phosphate buffer was adjusted accordingly. Initially, samples and 0.2 M phosphate buffer were added to tubes. The reaction mixture was then treated with 500 µL of potassium ferricyanide and then it was placed in the incubator for 20 minutes at 37ºC. The Eppendorf tubes were then filled with 500 µL of trichloracetic acid. Whole mixture was rotated at 3000 rpm in centrifuge for 10 minutes.

The 50 µL of the supernatant was transferred to an Eppendorf tube, followed by the addition of 100 µL of ferric chloride; and reaction mixture becomes blue color due to reduction of ferric chloride. This sample was divided into 200 µL and poured into wells. The samples' absorbance was then measured on a microplate reader at 630 nm. The reducing power of sample was calculated by:

Ascorbic Acid Equivalence = 100/2.7025 x Absorbance of sample µg/ ml

Stevia rebaudiana extract

From 2 different geographical locations in Pakistan plants sample were obtained and washed. Plants were then divided into the parts and dried. Then all the samples were grinded to form powder for further extraction and assays.

Aqueous extract of *S. rebaudiana*

The 10gm of samples was measured and then put into the beaker along with 100ml of distilled water and heated at 60ºC for 1 hour with continuous stirring and then cooled at roomtemperature and after that each sample was filtered by Whatman filter paper 42 and thencentrifuged to get the plant Aqueous Extract and then keep it in refrigerator for further uses (Aritonang, Koleangan et al. 2019).

Ethanolic extract of *S. rebaudiana*

The 10gm of each sample was taken and placed in a rotatory shaker with 450ml of 80%ethanol for 24 hours placed at room temperature then the extract was filtered by Wattman filter paper 42 and then centrifuged and kept in the refrigerator for assays (Romulo, Posner et al. 2018).

Total Flavonoid content Assay of *S. rebaudiana*

The plant extract can be calculated by colorimetric method (Chang, Yang et al. 2002) by using Aluminium chloride as a reagent. In this colorimetric method Quercetin is used as a standard compound and the plant extract is expressed graphicallyas Quercetin /gm of plant dried extract. The 1.0 ml of plant extract solution was placed in a test tube. Then 3ml of the methanol. 200-micro liter of 10% AlCl3 solution, 200 micro litter of 1M solution of potassium acetate, 5.6 ml of distilled water was added to extract solution subsequently. Then whole reaction mixture was incubated for 30 min for completion of the reaction. The absorbance of solution is calculated by using UV-Spectrophotometer.

The total flavonoid content of the extract is measured by equation

$$C = (c \times v) / m.$$

Whereas,

C=Total Flavonoid content, mg/g plant extract

c= Concentration of quercetion established from the calibration

v= Volume of the extract, ml

m= the weight of pure plants extract, gm

Stevia rebaudina leaves extract for HPLC

Grounded Stevia Leaves (10gm) were taken in flask with 25ml of acetonitrile water (8:2 v/v) instead of distilled water as used in other extraction technique and then heated at 102C for 30min and cooled at room temperature and filtered through membrane filter and then subjected for HPLC analysis (Woelwer-Rieck, Lankes et al. 2010).

Statistical analysis

The statistical analysis was done on all data obtained in MS Excel 2010.

Results and Discussion

Withania coagulans extract

Total phenolic content

It was resolute by folin-ciocaltue method and expressed as the Gallic acid equivalents. Concentration was expressed as the mg of GA/g of extract. After measuring absorbance at 730 nm, determination of phenolic content through calibration curve it was expressed in the Gallic acid equivalents. This can be seen in figure 1.

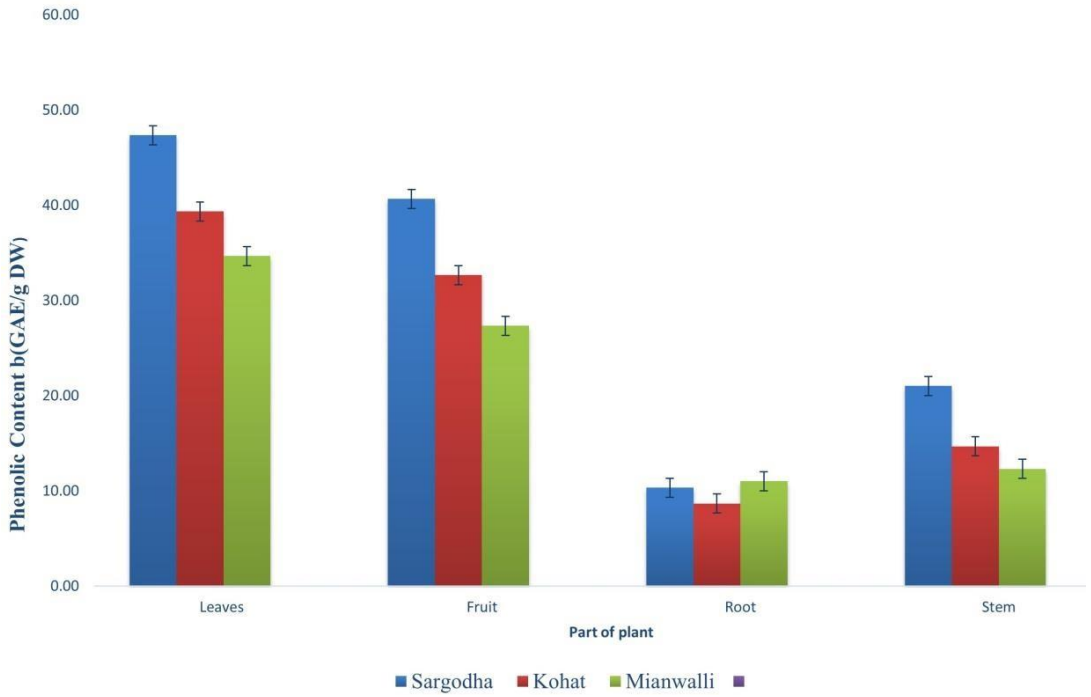


Figure 1: Indicates the total phenolic content in extracts

Total flavonoid Content Assay

It was measured by AlCl3 calorimetric method and expressed as Quercetin equivalents after measuring the absorbance of sample at 415 nm wavelength. Standard solution of Quercetin 0.0 to 25 µg/ml) was used to construct corresponding calibrating curve. The content was expressed as mg of Quercetin equivalents/g of extract. Flavonoid content was determined using Regression equation. This can be seen in figure 2.

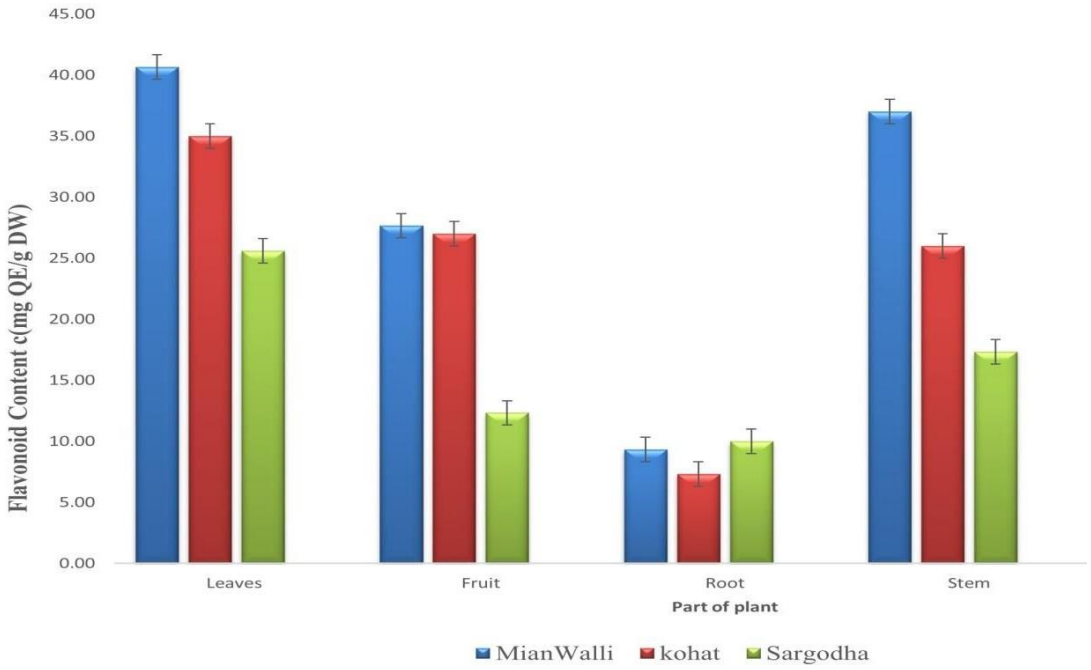


Figure 2: Indicates the flavonoid content in extracts

Reducing Power Assay

In this method yellow color of solution changes into different shades of blue and green developing upon the concentration of the reducing agent inside the reaction mixture when absorbance is measured at 630 nm. Reducing power of the different fractions was due to presence of the biological compounds that were electron donors in its nature. This is seen in figure 3.

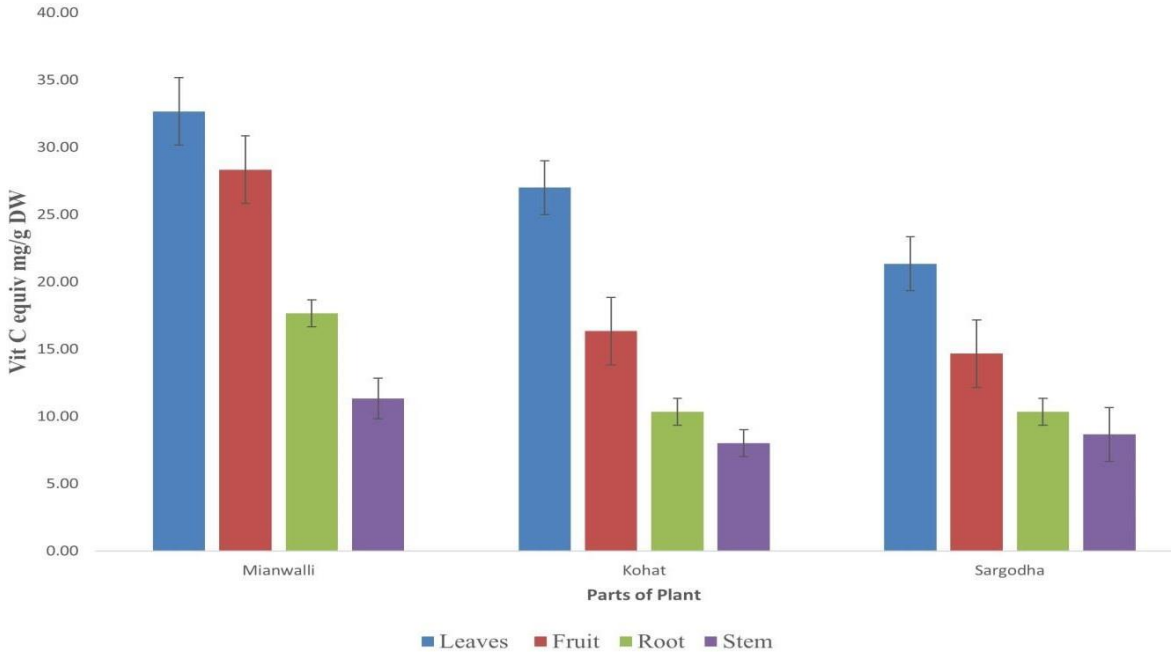


Figure 3: Shows the reducing power in extracts

Stevia rebaudiana **extract**

Total Flavonoid Content Analysis:

The total flavonoids (19.2±0.32, 22.9±0.18 and 13.79±0.17 mg Catechin/g) in methanolic extract, ethanolic extract and aqueous extract of (*S. rebaudiana)* leaves were found to be respectively. This is given in figure 4.

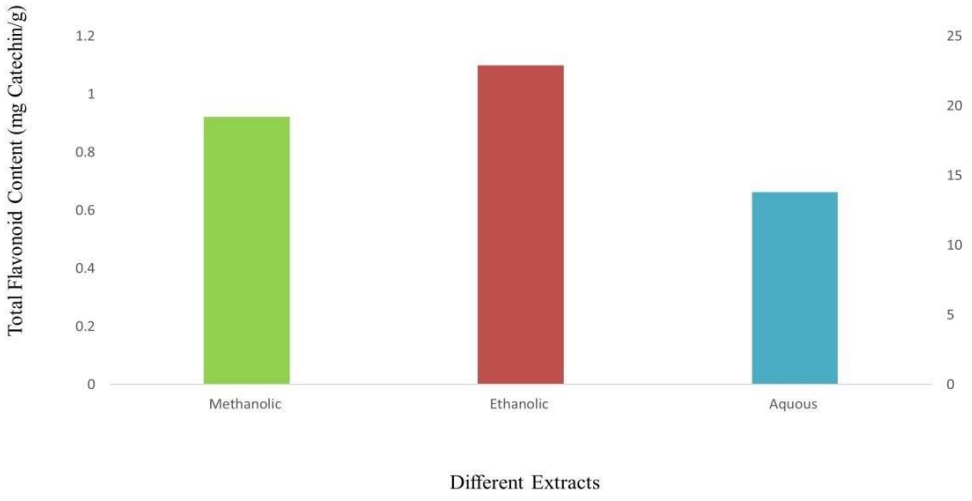


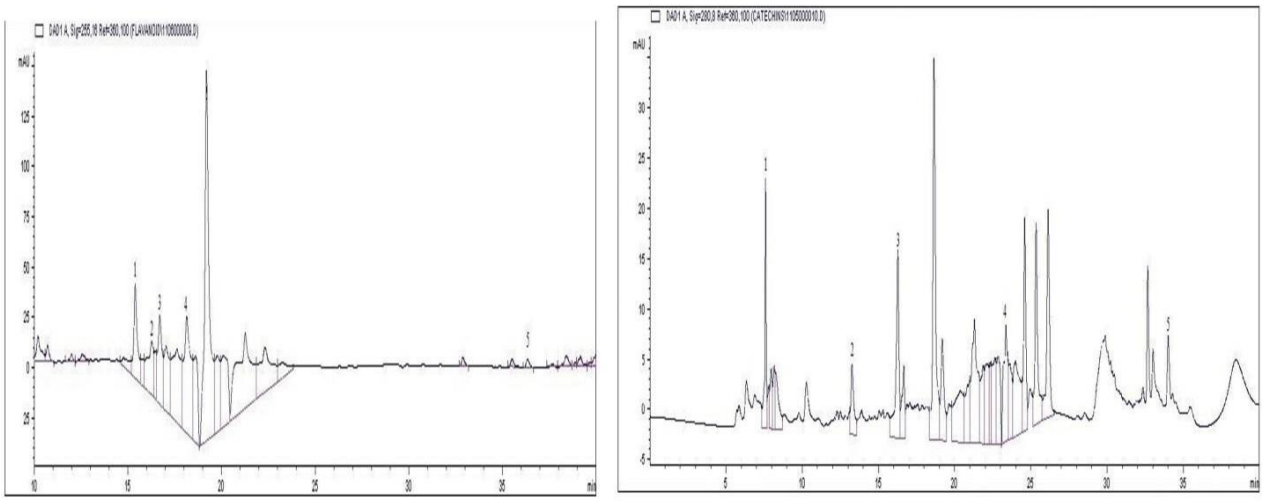
Figure 4: Indicates the total flavonoid content in different extracts

Quantification of Stevia leaves Extract by High performance Liquid Chromatography (HPLC)

Table 1 indicated the chemicals used for stevia leaves extraction are of standard grades >95% purity. Quantification of phytochemicals present in Stevia plant extract was obtained by (HPLC). Table 1 shows the level of phytochemicals present in the quantification by HPLC and it was found that by using wavelength of 255nm significant amount of rutin (0.14%) followed by quercetin-3-D- glycoside (0.05%) and luteolin (0.03%) was found. Catechins were quantified at wavelength 280nm and found that gallic acid (0.01%), epigallocatechin (0.72%), epicatechin (0.07%), gallocatechin (0.09%) and epicatechin gallate (0.03%). The figure 5 shows the HPLC reults.

Table 1: Quantification of Phytochemical at different wavelength

Phytochemicals	**Relative %**	**Wavelength**
Rutin	0.14%	255nm
quercetin-3-D-glycoside	0.05%)	
luteolin	0.03%)	
gallic acid	0.01%),	280nm
gallocatechin	0.09%),	
epigallocatechin	0.72%),	
epicatechin	0.07%)	
epicatechin gallate	0.03%)	



a) HPLC-chromatogram of flavonoids from S. rebaudiana leaves (wavelength 255 nm): 1- rutin, 2- hyperoside, 3- luteolin, 4- quercetin-3-D-glycoside, 5- kaempferol

b) HPLC-chromatograms of catechins from S. rebaudiana leaves (wavelength 280 nm): 1- gallic acid, 2- gallocatechin, 3- epigallocatechin, 4- epicatechin, 5- epicatechin gallate

Figure 5: Shows the HPLC chromatogram on leaves at different wavelengths

Discussion

The purpose of this work was to assess the antioxidant effect of medicinal plants including *W. coagulans* and *S. rebaudiana.*

The total phenolic content was found to be 47.33 ±2.50, 40.66 ±1.50, 10.33 ±2.00 and 21.00 ±2.00 (GAE/g DW) in leaves, fruit, root and stem respectively in Sargodha city. Kohat city has the highest phenolic content 39.33±1.50 (GAE/g DW) in the leaves while lowest 8.66 ± 2.50 (GAE/g DW) in the roots. In the same way leaves extract of Mianwalli city has the highest value of phenolic content (34.66 ±2.50) while lowest 11.00 ±2.50GAE/g DW) in the roots. Leaves of Sargodha have the highest value of phenolic content 47.33 ±2.50 (GAE/g DW) while Kohat city roots extract has the lowest phenolic content of 8.66 ± 2.50 (GAE/g DW). 58.21 mg GEA/g in leaves while 26.25 mg GEA/g and 15.95 mg GEA/g in stem and root respectively. In their study, the leaves of W. coagulans had a higher total phenolic contents value (58.21 mg GEA/g) compared to its stem and roots, which measured 26.25 mg GEA/g and 15.95 mg GEA/g, respectively. In W. somnifera, the TPC was also higher in the leaves (53.53 mg GEA/g), similar to the leaves of W. coagulans but slightly lower. These phenolic compounds in plants are crucial constituents known for their antioxidant properties (Khan, Maqsood et al. 2021).

The total flavonoid contents of Mianwalli city were found to be as 40.66 ±2.00, 27.6 ± 2,50, 9.33 ± 1.50 and 37.00 ±2.00mg QE/g DW) in leaves, fruit, root and stem respectively. Extract from the Kohat and Sargodha city has highest value of 35.00

±2.00, 25.60 ±3.05 and lowest value of 10.00± 1.0 and 7.00 ± 2.50 mg QE/g DW). In their study, they found the flavonoid content in W. coagulans leaves to be 47.0 mg RE/g, and in W. somnifera leaves to be 43.51 mg RE/g. There is a decreasing trend in flavonoid content from leaves to roots in both W. coagulans and W. somnifera, with the highest levels in the leaves and the lowest in the roots. The presence of flavonoids indicates the antioxidant activity of these plants. However, the concentration of flavonoids is significantly influenced by biological and genetic diversity, as well as environmental and temporal variations among different plants (Thiruppathi, Mohankumar et al. 2024).

Reducing power assay results for the Mianwali city were found to be 32.66±2.5, 28.33±2.5, 17.66±2.5 and 11.33±1.5 Vit C equiv mg/g DW in the leaves, fruits, root and stem respectively. While the Extract of Kohat and Sargodha city has 27.00±2.00, 16.33±2.50, 10.33±1.50, 8.00±1.00and 21.33±2.00, 14.66±2.50, 10.33±1.50, 8.66±2.00 Vit C equiv mg/g DW in the leaves, fruit, root and stem respectively. It was found that extract from Mianwalli city has highest value of reducing power of 32.66 ±2.5 while Sargodha city has lowest value of 8.66 ±2.00. Leaves of *W. coagulans* have highest value of reducing power 32.66 ±2.5 8.66±2.00 Vit C equiv mg/g DW while it gradually decreases in the fruits, roots and stem comparatively till 8.66±2.00 Vit C equiv mg/g DW. In their study (Akhtar 2022) the stem and leaves of W. coagulans and reducing power values were 38.77±2.2 and 25.7±3.7 Vit C equiv mg/g DW respectively. The reducing power assay results have the same pattern as the pattern repeated in the results of. Leaves have the highest values of reducing power compounds while stem has the lowest. Leaves obtained from the Mianwalli city have the highest value of reducing power assay while Sargodha has the least. Various studies have confirmed the existence of direct correlation among total phenolic, flavonoid, and antioxidant capacity (Aryal et al., 2019). These findings show that *W. coagulans Dunal* contain biomedical components that could be used in the formulation of modern medicines.

In this study, the total flavonoids (19.2±0.32, 22.9±0.18 and 13.79±0.17 mg Catechin/g) in methanolic extract, ethanolic extract and aqueous extracts of Stevia (*S. rebaudiana)* leaves were found respectively. In previous studies total Flavonoid content was reported by (Tadhani, Patel et al. 2007) and (Ghanta, Dutta et al. 2013) was 22.89mg GAE/g d.m and 0.84 mg QE/mg d.m by using the methanol and ethyl acetate solvent respectively in extraction technique. And another report total flavonol content found by (Kim and Jang 2011) was found to be 14.74 mg QE/g and 21.08 mg by using

water extract and methanol extract respectively. (Moongngarm, Sriharboot et al. 2022) reported that the total flavonol content in water and methanol extract was found to be 10.41 mg RE/g and 21.53 mg RE/g respectively. (Ngamsuk, Huang et al. 2019) reported that drying the stevia leaves at different temperature (30, 40,50 °C or above). The TPC and TFC measured in different procedure and found that the TFC was maximum at 40°C with TFC 139.07 mg QE/100 g than to fresh plant leaves. (Sarkar, Lahkar et al. 2022) found a positive correlation between the phytochemicals in plants and dehydrating the leaves and found that the in methanolic water extract of stevia leaves has increased phytochemicals on drying below 50 °C than to the phytochemicals in fresh leaves.

Conclusion

It is concluded that, total phenolic content was highest in Sargodha leaves (47.33 2.50 GAE/g DW) while Mianwali leaves extract had the greatest concentration of flavonoid QE/g DW (40.66 2.00 mg QE/g DW and reducing power of 32.662.5 Vit C equiv mg/g DW. While total flavonoid content for the stevia rebaudiana plant was found in good concentration in methanolic,ethanolic extract and aqueous extract of (*S. rebaudiana)* leaves.

Acknowledgment

Authors are thankful to the department for completing the work.

References

Admassu, S. and M. Kebede (2019). "Application of antioxidants in food processing industry: Options to improve the extraction yields and market value of natural products." Adv. Food Technol. Nutr. Sci **5**: 38-49.

Akhtar, M. A. (2022). "Anti-inflammatory medicinal plants of Bangladesh—a pharmacological evaluation." Frontiers in pharmacology **13**: 809324.

Amin, K., S. Ozgen and Z. Selamoglu (2017). "Stevia rebaudiana: A potential boon for human health." SM J Med Plants Stud **1**: 1005.

Aritonang, H. F., H. Koleangan and A. D. Wuntu (2019). "Synthesis of silver nanoparticles using aqueous extract of medicinal plants'(Impatiens balsamina and Lantana camara) fresh leaves and analysis of antimicrobial activity." International journal of microbiology **2019**(1): 8642303.

Azhar, S. C., A. Z. Aris, M. K. Yusoff, M. F. Ramli and H. Juahir (2015). "Classification of river water quality using multivariate analysis." Procedia Environmental Sciences **30**: 79-84.

Chanda, S. and R. Dave (2009). "In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview." African Journal of Microbiology Research **3**(13): 981-996.

Chang, C.-C., M.-H. Yang, H.-M. Wen and J.-C. Chern (2002). "Estimation of total flavonoid content in propolis by two complementary colorimetric methods." Journal of food and drug analysis **10**(3).

Ghanta, R., S. Dutta and R. Mukhopadhyay (2013). "Investigation on arbuscular mycorrhizal alliances in some threatened medicinal herbs of Burdwan district, West Bengal, India." Journal of Medicinal Plants Research **7**(7): 315-323.

Gupta, E., S. Purwar, S. Sundaram and G. Rai (2013). "Nutritional and therapeutic values of Stevia rebaudiana: A review." Journal of Medicinal Plants Research **7**(46): 3343-3353.

Gurunathan, S., M. Qasim, Y. Choi, J. T. Do, C. Park, K. Hong, J.-H. Kim and H. Song (2020). "Antiviral potential of nanoparticles—Can nanoparticles fight against coronaviruses?" Nanomaterials **10**(9): 1645.

Hacışevki, A. and B. Baba (2018). "An overview of melatonin as an antioxidant molecule: a biochemical approach." Melatonin molecular biology, clinical and pharmaceutical approaches **5**: 59-85.

Hosseinzadeh, S., A. Jafarikukhdan, A. Hosseini and R. Armand (2015). "The application of medicinal plants in traditional and modern medicine: a review of Thymus vulgaris." International Journal of Clinical Medicine **6**(09): 635-642.

Iatridis, N., A. Kougioumtzi, K. Vlataki, S. Papadaki and A. Magklara (2022). "Anti-cancer properties of Stevia rebaudiana; more than a sweetener." Molecules **27**(4): 1362.

Jahangir Chughtai, M. F., I. Pasha, T. Zahoor, A. Khaliq, S. Ahsan, Z. Wu, M. Nadeem, T. Mehmood, R. M. Amir and I. Yasmin (2020). "Nutritional and therapeutic perspectives of Stevia rebaudiana as emerging sweetener; a way forward for sweetener industry." CYTA-Journal of Food **18**(1): 164-177.

Khan, M. I., M. Maqsood, R. A. Saeed, A. Alam, A. Sahar, M. Kieliszek, A. Miecznikowski, H. S. Muzammil and R. M. Aadil (2021). "Phytochemistry, food application, and therapeutic potential of the medicinal plant (Withania coagulans): A review." Molecules **26**(22): 6881.

Kim, G. N. and H. D. Jang (2011). "Flavonol content in the water extract of the mulberry (Morus alba L.) leaf and their antioxidant capacities." Journal of Food Science **76**(6): C869-C873.

Kumar, S. and A. K. Pandey (2013). "Chemistry and biological activities of flavonoids: an overview." The scientific world journal **2013**(1): 162750.

Kurek, J. M. and Z. Krejpcio (2019). "The functional and health-promoting properties of Stevia rebaudiana Bertoni and its glycosides with special focus on the antidiabetic potential–A review." Journal of Functional Foods **61**: 103465.

Lemus-Mondaca, R., A. Vega-Gálvez, P. Rojas, K. Stucken, C. Delporte, G. Valenzuela-Barra, R. J. Jagus, M. V. Agüero and A. Pasten (2018). "Antioxidant, antimicrobial and anti-inflammatory potential of Stevia rebaudiana leaves: effect of different drying methods." Journal of Applied Research on Medicinal and Aromatic Plants **11**: 37-46.

Manjuladevi, R., R. Shilpa, R. Saratha, S. Selvasekarapandian and R. Mangalam (2019). Conductive bio-Polymer electrolyte with lithium salt for application in electrochemical device. AIP Conference Proceedings, AIP Publishing.

Maurya, P. K., J. Kalita and U. K. Misra (2010). "Spectrum of hypokalaemic periodic paralysis in a tertiary care centre in India." Postgraduate medical journal **86**(1022): 692-695.

Melguizo-Rodríguez, L., R. Illescas-Montes, V. J. Costela-Ruiz, J. Ramos-Torrecillas, E. de Luna-Bertos, O. García-Martínez and C. Ruiz (2021). "Antimicrobial properties of olive oil phenolic compounds and their regenerative capacity towards fibroblast cells." Journal of Tissue Viability **30**(3): 372-378.

Moongngarm, A., N. Sriharboot, P. Loypimai and T. Moontree (2022). "Ohmic heating-assisted water extraction of steviol glycosides and phytochemicals from Stevia rebaudiana leaves." Lwt **154**: 112798.

Mudassir, M. A., S. Z. Hussain, M. Khan, S. T. Asma, Z. Iqbal, Z. Huma, N. Ullah, H. Zhang, T. M. Ansari and I. Hussain (2018). "Polyacrylamide exotemplate-assisted synthesis of hierarchically porous nanostructured TiO 2 macrobeads for efficient photodegradation of organic dyes and microbes." RSC advances **8**(52): 29628-29636.

Ngamsuk, S., T.-C. Huang and J.-L. Hsu (2019). "Determination of phenolic compounds, procyanidins, and antioxidant activity in processed Coffea arabica L. leaves." Foods **8**(9): 389.

Ortiz-Viedma, J., N. Romero, L. Puente, K. Burgos, M. Toro, L. Ramirez, A. Rodriguez, J. Barros-Velazquez and S. P. Aubourg (2017). "Antioxidant and antimicrobial effects of stevia (Stevia rebaudiana Bert.) extracts during preservation of refrigerated salmon paste." European Journal of Lipid Science and Technology **119**(10): 1600467.

Pammi, S. S., B. Suresh and A. Giri (2023). "Antioxidant potential of medicinal plants." Journal of Crop Science and Biotechnology **26**(1): 13-26.

Peerzade, I. J., S. Mutturi and P. M. Halami (2024). "Improved production of RNA-inhibiting antimicrobial peptide by Bacillus licheniformis MCC 2514 facilitated by a genetic algorithm optimized medium." Bioprocess and Biosystems Engineering **47**(5): 683-695.

Ram, H., S. Sardar and N. Gandass (2021). "Vacuolar iron transporter (like) proteins: Regulators of cellular iron accumulation in plants." Physiologia Plantarum **171**(4): 823-832.

Romulo, C. L., S. Posner, S. Cousins, J. Hoyle Fair, D. E. Bennett, H. Huber-Stearns, R. C. Richards and R. I. McDonald (2018). "Global state and potential scope of investments in watershed services for large cities." Nature communications **9**(1): 4375.

Sarkar, R. D., P. Lahkar and M. C. Kalita (2022). "Glycosmis pentaphylla (Retz.) DC leaf extract mediated synthesis of selenium nanoparticle and investigation of its antibacterial activity against urinary tract pathogens." Bioresource Technology Reports **17**: 100894.

Sisubalan, N., S. Velmurugan, V. Malayaman, S. Thirupathy, M. H. G. Basha and R. R. Kumar (2014). "Ethnomedicinal studies on villages of Thenpuranadu, Tamil Nadu, India." Spatula DD **4**(1): 41-47.

Sofowora, A., E. Ogunbodede and A. Onayade (2013). "The role and place of medicinal plants in the strategies for disease prevention." African journal of traditional, complementary and alternative medicines **10**(5): 210-229.

Stanner, S., J. Hughes, C. Kelly and J. Buttriss (2004). "A review of the epidemiological evidence for the 'antioxidant hypothesis'." Public health nutrition **7**(3): 407-422.

Süntar, I. (2020). "Importance of ethnopharmacological studies in drug discovery: role of medicinal plants." Phytochemistry Reviews **19**(5): 1199-1209.

Sureshkumar, J., R. Silambarasan and M. Ayyanar (2017). "An ethnopharmacological analysis of medicinal plants used by the Adiyan community in Wayanad district of Kerala, India." European Journal of Integrative Medicine **12**: 60-73.

Tadhani, M., V. Patel and R. Subhash (2007). "In vitro antioxidant activities of Stevia rebaudiana leaves and callus." Journal of food composition and Analysis **20**(3-4): 323-329.

Thiruppathi, G., A. Mohankumar, D. Kalaiselvi, M. Velumani, P. Saravana Bhavan, P. Premasudha, S. Tawata and P. Sundararaj (2024). "Geroprotective Effect of Levilactobacillus brevis and Weizmannia coagulans in Caenorhabditis elegans." Probiotics and Antimicrobial Proteins **16**(2): 589-605.

Woelwer-Rieck, U., C. Lankes, A. Wawrzun and M. Wüst (2010). "Improved HPLC method for the evaluation of the major steviol glycosides in leaves of Stevia rebaudiana." European Food Research and Technology **231**: 581-588.

Wolfe, K., X. Wu and R. H. Liu (2003). "Antioxidant activity of apple peels." Journal of agricultural and food chemistry **51**(3): 609-614.