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A Review of Research on the Bioactivity of Stevia Rebaudiana Sp. (Phenolic and Flavonoid Compound Content and DPPH) in Morocco, Mexico, and Indonesia

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ABSTRACT

Stevia rebaudiana is a plant that contains many compounds with high antioxidant potential, but differences in activity are influenced by solvent factors and the growing environment. For this reason, this article review collects data on the types of solvents and the parts of the plant that can be used as the best sources for antioxidant activity. The method used in this literature review article (LRA) involves collecting and analyzing various journals that discuss the phytochemical and antioxidant activities of Stevia rebaudiana. Results from studies on the ethanol extract of S. rebaudiana leaves in Mexico show that the total phenolic content (TPC) in both Criolla and Morita varieties is lower than the total flavonoid content (TFC), while in Morocco, the TPC content is higher than the TFC. Based on DPPH test results, ethanol is the best solvent for extracting Moroccan Stevia, and the most suitable part of the plant to use is the leaves. The antioxidant activity varies across different regions. Furthermore, analysis of S. rebaudiana chromatogram varieties using HCA and PCA multivariate analysis can reveal the dominant marker and active compounds of the Stevia plant in each region.

KEYWORDS stevia, antioxidant, bioactivity

INTRODUCTION

The content of phytochemical compounds in herbal plants affects the efficacy of plant extracts and the overall quality of the plant. Stevia sweetener extract has a positive effect on human health, functioning as an antihypertensive, antihyperglycemic, non-kariogenic, antiviral, and supporting glucose metabolism and kidney function(Dewi et al., 2025; Raini & Isnawati, 2011; Zhafirah et al., 2024). Stevia leaves contain relatively high levels of phenolic compounds, vitamin C, carotenoids, and chlorophyll. In 2006, the World Health Organization (WHO) concluded that “stevioside and rebaudioside A are not genotoxic either in vitro or in vivo, and that the genotoxicity of stevioside and some of its oxidative derivatives in vitro does not occur in vivo”. Differences in plant composition caused by geographical conditions, extraction methods, and sample preparation techniques result in inconsistencies in findings across many studies on the chemical composition and biological activity of Stevia leaves (Parihar et al., 2020).

The varieties Stevia rebaudiana Bertoni Morita II and Criolla are obtained from gardens in the state of Yucatán, Mexico (Frankson et al., 2024). These plants are managed according to the production technology parameters established for Mexico (Ruiz et al., 2015). Stevia is a newly cultivated plant in Morocco. The antioxidant capacity of S. rebaudiana cultivated in Morocco had not previously been evaluated

when extracted using three different solvents (water, ethanol, and methanol), and studies have since classified and identified similarities and differences between the bioactive compounds of different varieties (Ibroham et al., 2022). In Indonesia, chromatographic fingerprint analysis of S. rebaudiana has been conducted on samples from various growing locations, leaf ages, and seedling origins. Similarity and quality classification tests were performed using Hierarchical Cluster Analysis (HCA) and Principal Component Analysis (PCA) chemometric methods, based on the retention times and areas of several HPLC chromatogram peaks. The results can be used for quality assurance and control of S. rebaudiana leaf raw materials.

Plant-derived antioxidants are a large group of bioactive compounds consisting of flavonoids, phenolic compounds, sulfur-containing compounds, tannins, alkaloids, phenolic diterpenes, and vitamins. Polyphenols are compounds containing aromatic rings and include phenolic acids, coumarins, flavonoids, stilbenes, and tannins (Iwuozor et al., 2024; Joshi, 2025; Mlambo et al., 2022; Papaefthimiou et al., 2024; Ruiz-Ruiz et al., 2017). The chemical properties of these compounds, along with solvent polarity, pose challenges in developing suitable procedures for extracting all polyphenols from plants. Various solvents such as water, acetone, methanol, ethanol, or mixtures of these with water have been used for polyphenol extraction. A solvent mixture consisting of ethanol (a type of alcohol safe for consumption) and water has proven to be suitable for extracting plant-derived phenolic compounds.

Total phenolic content (TPC) and total flavonoid content (TFC) are often used as rough indicators of antioxidant presence in plant extracts or plant-based food products (Cruz-Carrión et al., 2023; Djordjević et al., 2024). TPC is calculated using a standard curve, with results expressed in milligrams of gallic acid equivalent per gram of dry sample (mg GAE/g). TFC is determined based on the formation of an aluminum–flavonoid complex and is calculated using a standard curve in milligrams of equivalent flavonoid compounds such as quercetin, catechin, or rutin, which are commonly found in plants.

Several in vitro tests are used to measure the antioxidant activity of plant extracts, including the DPPH (2,2-diphenyl-1-picrylhydrazyl) test, the ABTS [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)] test, the FRAP (ferric reducing antioxidant power) test, and the ORAC (oxygen radical absorbance capacity) test. The DPPH assay provides a clear indication of the potential of bioactive compounds to combat oxidative stress. During extraction, antioxidant compounds react with DPPH, causing the solution to change from purple to yellow, a shift that can be measured quantitatively using a spectrophotometer. DPPH is an effective method for evaluating the ability of extracts to neutralize free radicals. This study is a review of research journals on Stevia bioactivity from various countries, including Morocco, Mexico, and Indonesia.

This article aims to collect, analyze, and synthesize existing research on the bioactivity of Stevia rebaudiana from Morocco, Mexico, and Indonesia. The primary objectives are: (1) to compare the Total Phenolic Content (TPC) and Total Flavonoid

Content (TFC) of Stevia extracts from the three countries; (2) to evaluate the influence of different extraction solvents on antioxidant activity (measured by the DPPH assay) of Stevia from these regions; (3) to identify the optimal plant part for maximizing antioxidant yield; and (4) to discuss the utility of chemometric techniques such as HCA and PCA in standardizing Stevia raw materials. The benefits of this research are twofold. Academically, it provides a comprehensive overview to inform future research design. Practically, it offers valuable insights for the nutraceutical and food industries in selecting raw materials and developing standardized extraction protocols to ensure consistent quality and efficacy of Stevia-based products.

RESEARCH METHOD

Material

This research uses materials from research journal library searches, scientific articles and journal reviews through electronic databases such as Google Scholar, PubMed and ScienceDirect. Search and search of the literature was carried out using related keywords such as: stevia, anthiooxidant, biodiversity. The data sources that can be obtained consist of international journals as the main data source and national journals as additional data sourcesThe search for articles and journals was carried out from January 2025 to November 2025 (Papaefthimiou et al., 2024).

Method

The method used in this review is *literature review article* (LRA) by collecting and analyzing various journals that discuss the phytochemical and antioxidant activity of stevia from it. The stevia leaves in the Mexican study were extracted with a methanol solvent (200 mL) using a soxhlet tool so that the solution in the tube became clear. In Morocco air-dried stevia leaves (10 g) are mashed, then extracted with 100 ml of methanol, ethanol and aqueous solution through a maceration process. From the results of a study comparing the solvents used, extraction using ethanol solvents produced the highest gain but provided the lowest levels of steviosides, inversely proportional to aquaade distillate solvents.

The total phenolic content (TPC) was determined using the Folin-Ciocalteu (F-C) test. The calculation of the total flavonoid content (TFC) is based on the formation of an aluminum-flavonoid complex. A total of 0.5 ml of DPPH solution was dissolved in 4.5 ml of methanol, then 10 µl of extract was added. A control without extracts is also maintained. The mixture is shaken and then left for 45 minutes in the dark. Absorbance is measured at 515 nm. Highly stable organic free radicals with a dark purple color that provides maximum absorbance in the range of 515-528 nm (2).

RESULTS AND DISCUSSION

This research process focuses on collecting existing data and information through literature review and analysis from published journals. In addition, the study involved screening and antioxidant activity to evaluate the content phytochemicals and their potential antioxidant activity in stevia leaves. Stevia that has been extracted by

different methods is assessed for yield quality and checked for Phytochemical Screening. Phytochemical screening of stevia leaves was carried out qualitatively to see for the presence of flavonoids, saponins, quinones, coumarins, phenolics, tannins, and terpenoids (9). From the sample materials tested, each region was the same from the leaf extract but had a different geographical aspect of plant origin so that it affected the content and nutrients in stevia leaves. These differences in cultivation locations can result in diverse types and amounts of secondary metabolite compounds.

Table 1. Phytochemical ScreeningS.rebaudiana Bertoni Indonesia

Phytophytomy Content	**Interpretation**	**Function**
Flavonoids	Dark Yellow	anti-inflammatory, antidiabetic, cardio- and neuro-protective, as well as slowing down the aging process (Dias et al. 2021)
Saponins	Foam	immunomodulators, anti-inflammatory, and antitumors (Yang et al. in 2021).
Quinon	Red	antiviral and antiparasitic (Tong et al. Year 2022; Yang et al. Year 2020).
Squirrelly	Yellow Fluorescence	antiviral and antiparasitic (Tong et al. Year 2022; Yang et al. Year 2020).
Phenolics	Dark Green	Anti-cancer, cardiovascular disease, diabetes, osteoporosis, and neurological disorders (Rahman et al. 2021).
Tannins	Blackish green	Antiviral and antiparasitic

		that he has (Tong et al. in 2022; That et al. in 2020)
Terpenoids	Brown	antiviral and antiparasitic properties (Tong et al. 2022; That et al. in 2020)

The study in Morocco planted seeds in six regions in Morocco in the same month, namely Agadir, Berkane, Larache, Marrakech, Rabat, and Sefrou. These regions have different ecological characteristics. Research in Mexico of *Stevia rebaudiana* Bertoni Morita II and criolla varieties obtained from orchards in the State of Yucatan, Mexico. Meanwhile, the leaves of *S. rebaudiana* Indonesia came from the highlands of Bandungan, Magelang Regency, Central Java, obtained from the P.T Java Sakti Niaga plantation, and from local farmers Tawangmangu. S. rebaudiana leaves come from various growing places with a height ranging from 800 – 1400 m above sea level, variations in the age of harvested leaves from 5 days to 4 months are calculated based on the growing age of the plant, and variations in the origin of seedlings. The number of sample variations was 20.

Determination *of Total Phenolate Capacity* (TFC)

The content of phenolic compounds was tested using the Folin-Ciocalteu method, based on the reduction of phosphorus-wolframat-phosphomolybdate complexes by phenolic compounds into blue reaction products. One gram of the sample was dissolved in 40 mL of 80% methanol (v/v) in a beaker (Gupta et al., 2018; Neupane & Lamichhane, 2020; Pallab et al., 2013). The dispersion is stirred on a magnetic plate at room temperature for 3 hours. After extraction, disperse in centrifugation at 2500 rpm for 20 minutes at 10°C. The supernatant is filtered, cooled to 4°C, and protected from light until analysis. A total of 0.2 mL of methanol extract was placed in a plastic cell for a spectrophotometer, 0.2 mL of Folin-Ciocalteu reagent was added, and homogenized. Then 2 mL of distilled water is added, and the cells are kept in the dark at room temperature for 1 hour. Absorbance is measured at 765 nm. Data were calculated by comparing the standard curve (0–500 μg/mL of gallic acid) with the absorbance of each sample. Analysis was performed on three replications. The total number of phenolic compounds is determined in micrograms of gallic acid equivalent/mg of sample.

Phenolic compounds are secondary metabolites consisting of benzene rings with hydroxyl substituents, which are able to act as antioxidants. In the total phenolic level test, the standard curve of the galic acid concentration with the equation y=0.0009x+0.0053. The phenolic content is greater than 1 mg of GAE/g DW, so it can be said that stevia leaf extract has a relatively high phenolic content. The total phenolic

compound content measured in different extracts from samples originating from different geographical regions in Morocco was found to range from 37.13 to 67.85 mg of galic acid per gram of dry weight of stevia leaves in water extracts with the order Agadir, Larache, Rabat, Marrakech, Sefrou, and then Berkane. The data on the total phenolate content of ethanol and methanol extracts showed significant variation between regions, with a range of 27.56 to 47.77 mg/g, and 25.39 to 43.45 mg/g dw. The total phenolate content in stevia leaves from different regions shows significant differences. Agadir has the highest value in total phenolic content compared to six other Moroccan varieties.

Table 2. Phytochemical Content *of Stevia rebaudiana* in Morocco and Mexico

Parameters	Creole	Moritta II	Agadir	Sefrou
TPC (GAE/g DW)	28.7	28.4	27.6	47.8
TFC (mg quercetin equivalents/g)	39.3	36.7	19.87	33.86

The data are presented as an average (n = 3). Different letters showed significant differences ($P < 0.05$).

From table 2, it appears that the ethanol extract of S. rebaudiana leaves in Mexico is lower than the total phenolate content than the flavonoid content, while for Morocco the TPC content is higher than TFC.

Determination *of Total Flavonoid Capacity* (TFC)

The flavonoid content is determined using the aluminum chloride method. 0.5 mL of ethanol extract was placed in a plastic cell for a spectrophotometer, then 1.5 mL of 95% ethanol, 0.1 mL of 10 mL of $AlCl_3$, 0.1 mL of 1.0 M potassium acetate, and 2.8 mL of distilled water were added. The cells are stored in dark conditions at room temperature for 30 minutes. Absorbance is measured at a wavelength of 415 nm. Data were calculated by comparing the standard curve (0–100 μg/mL of quercetin) with the absorbance of each sample. Analysis was performed on three replications. The total number of flavonoid compounds is determined in micrograms of quercetin equivalent per milligram of sample.

The flavonoid content varies from 33.31 to 50.04 mg rutin/g, 19.87 to 33.86 mg/g, and 18.92 to 27.03 mg/g dw, respectively for stevia leaf water, ethanol, and methanol extracts. The results of the total phenolate compound content and flavonoids show that water extract has the highest content compared to ethanol and methanol extracts. The total number of flavonoids taken from different samples of *S. rebaudiana* leaves is in the following order: Agadir, Larache, Rabat, Marrakech, Berkane, Sefrou. The total flavonoid content in *S. rebaudiana* leaves shows significant differences between regions and regions. In a 2005 study by Akowuah and Kawan Kawan in

Indonesia, it was stated that the total phenolate and flavonoid content of plants is greatly influenced by several factors, including: the geographical location or origin of the plant, the nutrient content in the soil where the plant grows, and the type of plant.

Antioxidant activity of Stevia rebaudiana extract

Based on the June 2003 study, IC50 values were grouped into 5, namely very strong with IC50 less than 50 μg/mL, strong in IC50 between 50-100 μg/mL, medium between 101-250 μg/mL, weak in the range of 251-500 μg/mL and very weak (inactive) in IC50 more than 500 μg/mL. The DPPH test was measured with a spectrophotometer, the reducing effect of antioxidants against the 2,2-diphenylpicrylhydrazyl (DPPH) radical. Radical arrest activity of DPPH in *S. rebaudiana* Bertoni extract. Antioxidant activity shows a direct dose-dependent correlation with extract concentration.

From a study in Morocco, it is known that the IC50 value of DPPH radical inhibition with stevia leaf water extract differs significantly ($P<0.05$) from ethanol and methanol extracts. Stevia Agadir has the highest TPC and TFC content and has the strongest DPPH inhibition, followed by Larache, Rabat, Marrakech, Berkane, and Sefrou respectively. A better solvent used to extract Moroccan Stevia in the DPPH test is ethanol as shown in table 3.

Table 3. Stevia Research Data With Various Solvents in Morocco (2)

SOLVENT NAME	VALUE IC50 in Sefrou	IC50 Agadir VALUE
WATER	69.01 %	78.08%
ETHANOL	59.56 %	66.42%
METHANOL	61.90 %	68.53%

Singh and colleagues in Mexico evaluated the antioxidant activity of methanol extracts from different parts of *the Stevia rebaudiana plant* (roots, stems, leaves, and flowers) and the activity of.

The reported capture of free radicals (%) ranged from 47.1% for leaves and 82.4% for flowers, respectively. This means that the right material to see the antioxidant activity of stevia on DPPH is the leaf. Research in Indonesia conducted a similarity test and classification of the quality of S. rebaudiana herbal plants by Hierarchical Cluster Analysis (HCA) and Principal Component Analysis (PCA) chemometrics based on retention time and area of several peak KCKT chromatograms.

Analysis of S. rebaudiana chromatogram variety with HCA multivariate analysis gave 5 marker peaks, namely peaks no 1, 2, 4, 6 and 7 where peaks no 6 and 7 are the dominant active compounds rebaudioside A and steviosides, respectively. The results of the PCA classification can directly group S. rebaudiana leaf samples based on the characteristics of the origin of the seedlings, the age of the leaves and the area of planting stevia seedlings.

CONCLUSION

Based on studies from several journals reviewed, it can be analyzed that the bioactivity of stevia leaf extract is different due to the difference in solvents used for extraction, the part of the plant used as the research sample material and the area where the stevia plant seeds are planted. Analysis of *S. rebaudiana* chromatogram variegated fingerprints with HCA and PCA multivariate analysis can inform the dominant marker and active compounds of stevia plants in each region.

REFERENCE

Cruz-Carrión, Á., Ruiz de Azua, M. J., Muguerza, B., Mulero, M., Bravo, F. I., Arola-Arnal, A., & Suarez, M. (2023). Organic vs. non-organic plant-based foods—A comparative study on phenolic content and antioxidant capacity. *Plants*, *12*(1), 183.

Dewi, A. C., Hadyanawati, A. A., Sarinindiyanti, J. A. A. N., & Putra, H. (2025). Pemanfaatan tanaman stevia sebagai pemanis alami pengganti gula. *Ikra-Ith Abdimas*, *9*(2), 25–32.

Djordjević, M., Djordjević, M., Starowicz, M., & Krupa-Kozak, U. (2024). Plant-based antioxidants in gluten-free bread production: Sources, technological and sensory aspects, enhancing strategies and constraints. *Antioxidants*, *13*(2), 142.

Frankson, D., Hendrawan, S., & Ferdinal, F. (2024). Evaluation of phytochemical content, antioxidant capacity, and toxicity of *Stevia rebaudiana* leaf extract. *Biosains: Jurnal Ilmiah Biologi*, *12*(2), 1622–1635.

Gupta, S., Singh, A., Dikshit, H. K., Aski, M., Mishra, G. P., & Kumar, J. (2018). Assessment of total phenol content, total flavonoid content and anti-oxidant capacity in exotic lentil germplasm. *Chemical Science Review and Letters*, *7*, 459–463.

Ibroham, M., Jamilatun, S., & Ika, D. K. (2022). A review: The potential of plants in Indonesia as natural antioxidants. *Seminar Nasional Peneliti LPPM UMJ*, 1–13. Retrieved from http://jurnal.umj.ac.id/index.php/semnaslit

Iwuozor, K. O., Emmanuel, S. S., Ahmed, M. O., Idris, A. M., Emenike, E. C., Saliu, O. D., Qudus, A. H., & Adeniyi, A. G. (2024). Technologies for the extraction and post-extraction of *Stevia rebaudiana* leaves. *Chemistry Africa*, *7*(2), 539–563.

Joshi, J. R. (2025). *Extraction of stevioside and rebaudioside A from Stevia rebaudiana Bertoni leaves* (Unpublished thesis). Gujarat Technological University.

Khiraoui, A., Al Faiz, C., Hasib, A., Bakha, M., Benhmimou, A., Amchra, F. Z., et al. (2018). Antioxidant ability, total phenolic and flavonoid contents of leaf extract of *Stevia rebaudiana* Bertoni cultivated in Morocco. *International Journal of Scientific & Engineering Research*, *9*(5), 1585–1589.

Martono, Y., Riyanto, S., Martono, S., & Rohman, A. (2016). Fingerprint analysis of *Stevia rebaudiana* using hierarchical cluster analysis (HCA) and principal component analysis (PCA). *Journal of Analytical Science and Technology*, *21*(1), 30–37.

Mlambo, R., Wang, J., & Chen, C. (2022). *Stevia rebaudiana*, a versatile food ingredient: The chemical composition and medicinal properties. *Journal of Nanomaterials*, *2022*(1), 3573005.

Neupane, P., & Lamichhane, J. (2020). Estimation of total phenolic content, total flavonoid content and antioxidant capacities of five medicinal plants from Nepal. *Vegetos*, *33*(2), 360–366.

Papaefthimiou, M., Kontou, P. I., Bagos, P. G., & Braliou, G. G. (2024). Integration of antioxidant activity assays data of stevia leaf extracts: A systematic review and meta-analysis. *Antioxidants*, *13*(6), 692.

Pallab, K., Tapan, B., Tapas, P., & Ramen, K. (2013). Estimation of total flavonoids content (TPC) and antioxidant activities of methanolic whole plant extract of *Biophytum sensitivum* Linn. *Journal of Drug Delivery and Therapeutics*, *3*(4), 33–37.

Parihar, N., Saini, M., Soni, S. L., & Sharma, V. (2020). Emulgel: A topical preparation. *Asian Journal of Pharmaceutical Research and Development*, *8*(3), 196–201.

Raini, M., & Isnawati, A. (2011). Kajian: Khasiat dan keamanan stevia sebagai pemanis pengganti gula. *Media Penelitian dan Pengembangan Kesehatan*, *21*(4).

Ruiz, J. C. R., Ordoñez, Y. B. M., Basto, Á. M., & Campos, M. R. S. (2015). Antioxidant capacity of leaf extracts of two varieties of *Stevia rebaudiana* Bertoni adapted to cultivation in Mexico. *Nutrición Hospitalaria*, *31*(3), 1163–1170.

Ruiz-Ruiz, J. C., Moguel-Ordoñez, Y. B., & Segura-Campos, M. R. (2017). Biological activity of *Stevia rebaudiana* Bertoni and their relationship to health. *Critical Reviews in Food Science and Nutrition*, *57*(12), 2680–2690.

Zhafirah, N., Lesmana, D., & Kintawati, S. (2024). Efek konsumsi stevia pada pH saliva kelompok dewasa muda: Tinjauan literatur. *Stomatognatic: Jurnal Kedokteran Gigi*, *21*(2), 94–101.