

Antibacterial effects of steviol glucoside extract from *stevia rebaudiana* grown in vivo in the Mediterranean climate of northwestern Algeria

Efeitos antibacterianos do extrato de glicosídeo de esteviol de *stevia rebaudiana* cultivada in vivo sob clima mediterrâneo Noroeste da Argélia

Efectos antibacterianos del extracto de glucósido de esteviol de *stevia rebaudiana* cultivado in vivo en el clima mediterráneo del noroeste de Argelia

DOI: 10.34188/bjaerv8n1-080

Submetido: 02/12/2024

Aprovado: 15/01/2025

Hanane Belhadj

Higher School of Biological Sciences of Oran, PB 1042 SAIM Mohamed, Emir Abdelkader City (EX-INESSMO) 31003 Oran, Algeria

Toxicology, Environment and Health Laboratory, Faculty of Natural and Life Sciences, University of Science and Technology of Oran - Mohamed

Oran. Algeria

E-mail: belhadj.hananemb@yahoo.fr

Abbassia Ayache

Plant Biodiversity: Conservation and Valorization Laboratory. Djillali Liabes University of Sidi Bel Abbes

Sidi Bel Abbes. Algeria

E-mail: abayache4@gmail.com

Belaidouni Meriem

Higher School of Biological Sciences of Oran, PB 1042 SAIM Mohamed, Emir Abdelkader City (EX-INESSMO)

Oran. Algeria

E-mail: meriembelaidouni@gmail.com

Benmeddah Fatiha

Higher School of Biological Sciences of Oran, PB 1042 SAIM Mohamed, Emir Abdelkader City (EX-INESSMO)

Oran. Algeria

E-mail: benmeddahfatiha3@gmail.com

Sahraoui Kamel

Higher School of Biological Sciences of Oran, PB 1042 SAIM Mohamed, Emir Abdelkader City (EX-INESSMO)

Oran. Algeria

E-mail: kamelsahraoui1300@gmail.com

Amieur Hind

Higher School of Biological Sciences of Oran, PB 1042 SAIM Mohamed, Emir Abdelkader City (EX-INESSMO)

Oran. Algeria

E-mail: hinddjihadamieur@gmail.com

Berebah Alioua Amel

Toxicology, Environment and Health Laboratory, Faculty of Natural and Life Sciences, University of Science and Technology of Oran - Mohamed Boudiaf
Oran. Algeria
E-mail: amelalioua2000@yahoo.fr

ABSTRACT

A phytochemical study was carried on seeds grown from *Stevia rebaudiana* in the Northwestern Algeria. The main objective of this research is to study the improvement of the growing conditions of these seeds on our lands. On the other hand, a screening, a study of their antioxidant activity, the extraction and evaluation of the antibacterial activity of steviol glycoside were carried to evaluate its therapeutic benefits. These virtues are used in the treatment of: inflammation, type two diabetes, cardiovascular diseases, gastrointestinal and dermatological problems. The recycled virtues of this species can be used as organic fertilizer. These experiments revealed that the selected *Stevia rebaudiana* seeds, aged five years, germinated better than those aged three years. A three-centimeter-high transplant of the young plants was carried at the right time. The growth of *Stevia* accelerated and its height reached up to forty centimeters. The phytochemical screening of the leaves of *Stevia rebaudiana* shows its richness in several compounds such as: alkaloids, flavonoids, tannin and saponin. The antioxidant activity results demonstrated the best activity. Extraction and purification of steviol, glycosides, including conventional extractions. After liquid-liquid purification, the decoction method gave the best yield. Steviol glycoside extract has inhibitory activity against *Staphylococcus aureus* and *Bacillus Cereus* with slightly inhibitory activity against *Pseudomonas aeruginosa* and *Escherichia coli*. However, no effects were observed on *Klebsiella Pneumoniae*. These results reveal that the climate of north of Algeria is also favorable for the cultivation of *Stevia*. The abundance of these active ingredients gives the plant remarkable pharmacological properties.

Keywords: phytochemical, stevia rebaudiana, antibacterial, steviol glycoside, cultivation of *Stevia*.

RESUMO

Foi realizado um estudo fitoquímico em sementes cultivadas de *Stevia rebaudiana* no noroeste da Argélia. O objetivo principal desta pesquisa é estudar a melhoria das condições de cultivo dessas sementes em nossas terras. Por outro lado, foi realizado um rastreio, um estudo da sua atividade antioxidante, a extração e avaliação da atividade antibacteriana do glicosídeo de esteviol para avaliar os seus benefícios terapêuticos. Essas virtudes são utilizadas no tratamento de: inflamações, diabetes tipo dois, doenças cardiovasculares, problemas gastrointestinais e dermatológicos. As virtudes recicladas desta espécie podem ser utilizadas como fertilizante orgânico. Esses experimentos revelaram que as sementes de *Stevia rebaudiana* selecionadas, com idade de cinco anos, germinaram melhor do que aquelas com idade de três anos. Um transplante de três centímetros de altura das plantas jovens foi realizado no momento certo. O crescimento da *Stevia* se acelerou e sua altura chegou a quarenta centímetros. A triagem fitoquímica das folhas de *Stevia rebaudiana* mostra sua riqueza em diversos compostos como: alcalóides, flavonóides, taninos e saponinas. Os resultados de atividade antioxidante demonstraram a melhor atividade. Extração e purificação de esteviol, glicosídeos, incluindo extrações convencionais. Após a purificação líquido-líquido, o método de decocção apresentou o melhor rendimento. O extrato de glicosídeo de esteviol possui atividade inibitória contra *Staphylococcus aureus* e *Bacillus Cereus* com atividade levemente inibitória contra *Pseudomonas aeruginosa* e *Escherichia coli*. No entanto, não foram observados efeitos na *Klebsiella Pneumoniae*. Estes resultados revelam que o clima do norte da Argélia também é favorável ao cultivo de *Stevia*. A abundância destes princípios ativos confere à planta propriedades farmacológicas notáveis.

Palavras-chave: fitoquímico, stevia rebaudiana, antibacteriano, glicosídeo de esteviol, cultivo de stevia.

RESUMEN

Se llevó a cabo un estudio fitoquímico en semillas cultivadas de *Stevia rebaudiana* en el noroeste de Argelia. El principal objetivo de esta investigación es estudiar la mejora de las condiciones de cultivo de estas semillas en nuestras tierras. Por otro lado, se realizó un screening, un estudio de su actividad antioxidante, la extracción y evaluación de la actividad antibacteriana del glucósido de esteviol para evaluar sus beneficios terapéuticos. Estas virtudes se utilizan en el tratamiento de: inflamación, diabetes tipo dos, enfermedades cardiovasculares, problemas gastrointestinales y dermatológicos. Las virtudes recicladas de esta especie se pueden utilizar como abono orgánico. Estos experimentos revelaron que las semillas seleccionadas de *Stevia rebaudiana*, de cinco años de edad, germinaban mejor que las de tres años. En el momento adecuado se realizó un trasplante de las plantas jóvenes a una altura de tres centímetros. El crecimiento de la *Stevia* se aceleró y su altura alcanzó hasta los cuarenta centímetros. El análisis fitoquímico de las hojas de *Stevia rebaudiana* muestra su riqueza en varios compuestos como: alcaloides, flavonoides, taninos y saponinas. Los resultados de actividad antioxidante demostraron la mejor actividad. Extracción y purificación de esteviol, glucósidos, incluidas extracciones convencionales. Después de la purificación líquido-líquido, el método de decocción dio el mejor rendimiento. El extracto de glucósido de esteviol tiene actividad inhibitoria contra *Staphylococcus aureus* y *Bacillus Cereus* con actividad ligeramente inhibitoria contra *Pseudomonas aeruginosa* y *Escherichia coli*. Sin embargo, no se observaron efectos sobre *Klebsiella Pneumoniae*. Estos resultados revelan que el clima del norte de Argelia también es favorable para el cultivo de *Stevia*. La abundancia de estos principios activos confiere a la planta notables propiedades farmacológicas.

Palabras clave: fitoquímico, *stevia rebaudiana*, antibacteriano, glucósido de esteviol, cultivo de *stevia*.

1 INTRODUCTION

The diet is often too rich in lipids and high-calorie carbohydrates, excessive consumption of which most often causes irreversible damage marked by obesity and an increase in the risk of diabetes in the more or less long term (Waqas et al. 2017, Kacper et al. 2022). Diabetes is the direct cause of 1.6 million deaths worldwide. The World Health Organization said in 2016 that diabetes was the direct cause of 1.6 million deaths worldwide. It is obvious that the main factor in these two epidemics and others is an poorly balanced diet. Researchers at Harvard University have shown that more than half of the global burden of disease is the result of poor diet (lack, excess or imbalance) (Venkat et al. 2011; Wang et al. 2019). In Algeria, the average sugar consumption reaches 30 kg per year per person, three times the standard recommended by the World Health Organization. This overconsumption of sugar has increased the number of diabetics in our country to more than 4 million. (Lamri et al. 2014, World Health Organization, 2012). In order to reduce these two major problems; and offering the sweet taste but without its drawbacks (Deliza et al. 2021, Wilk et al. 2022). Food industries are currently showing great interest in the use of low-calorie sweeteners instead of sugars and derivatives (Castro-Muñoz et al. 2022). Sweeteners are natural or synthetic molecules such as aspartame, acesulfame K, saccharin or even cyclamate, with sweetening properties which provide few or no calories when ingested instead of sugars and derivatives (Edwards and al. In addition, synthetic sweeteners are perceived by public opinion as chemicals that

can be harmful to health after several years of consumption despite their current use and consumers are seeking to return to natural products to become an alternative in order to produce (Light) or low sugar. high-calorie (healthier) foods (Chen et al. 2023, Pang et al. 2021). In this regard, “Stevia” is perhaps best known as an alternative to sugars. It is a genus of approximately 240 species in the Asteraceae family, named *Stevia rebaudiana* (Bertoni) (Yadav et al. 2011). It appeared on the edges of the subtropical forest of northeastern Paraguay, with sweet-tasting leaves, because it has been used by the Guarani Indians for several centuries to sweeten their traditional drink and treat various diseases and conditions (Ramesh et al. 2006). This plant was discovered by Europeans in 1887 and was later studied chemically. It has sweetening molecules which are glycosylated diterpenes, also called steviol glycosides, the main ones of which are rebaudioside A and stevioside with very high sweetening power and are non-caloric (nearly 300 times sweeter than sucrose) (Ramos- Tovar et al. 2019, Liu et al. The use of Stevia expanded in Japan from the late 1990s. 1970 (Ashwell, 2015), it represented 40% of the intense sweetener market in this country (annual consumption estimated at 200 t.) (Leth et al. 2007). Some studies have reported that this plant “Stevia” has confederate effects: analgesic, antimicrobial, antifungal, antioxidant, hypotensive effects and digestive disorders (Borgo et al. 2021). Over the following decades, the cultivation of *Stevia rebaudiana* expanded in many countries such as Paraguay, Brazil, Uruguay and Central America. China, Thailand, India and Algeria have also launched commercial crops (Hossain et al. 2017, Nazrul et al. 2020). The aim of this research is to take a critical look at the possibility of cultivating this plant in the Mediterranean climate as well as its biochemical properties and its therapeutic interest.

2 MATERIALS AND METHODS

2.1 DESCRIPTION OF THE STUDY AREA

Stevia is very attractive in Ghardaïa, particularly in the Oued Metlili area, from where we brought our plant material. The province of Ghardaïa is limited on the north by the province of Laghouat (200 km), to the east by the province of Ouargla (200 km), to the southwest by the province of Adrar (400 km) and to the West by the province of El-Bayad (350 km) (Arabi et al. 2021)(figure 1). The province of Ghardaïa has great agricultural potential, its soils are very fertile, and the subsoil contains significant water resources as well as its xeric Saharan climatic conditions constitute a natural biodiversity of the Saharan flora (Khaled et al. 2020). Our sample comes from the locality of Oued Metlili. It takes its name from the Wadi that crosses it. These Wadis were for a long time the only water resource of the Oases. The territory of Oued Metlili is located 40 km south of Ghardaïa, it is located between the following geographical coordinates: 32° 16' 22'' North, 3°

37° 39'' East. Metlili has a hot desert climate, with very hot summers and mild winters, and very little precipitation.

2.2 SEEDS COLLECTION

The collection of seeds was carried out directly from the plants of *S. Rebaudiana* from the cultivation fields of Oued Metlili, Ghardaïa (south-east of Algeria). A batch of unselected seeds (2019 collection) were kept in paper bags, provided with a label with the name of the specie and the location.

Figure 1. Geographical location of the study area by satellite view of (google earth 2024). The locality of Oued Metlili presents a hot desert bioclimate, with very hot summers and mild winters, and very little precipitation. *Stevia rebaudiana* is found in these natural environments on the edge of wadis, with soils with shallow water tables, continually wet but not subject to prolonged flooding. Source: Prepared by the authors (2024).

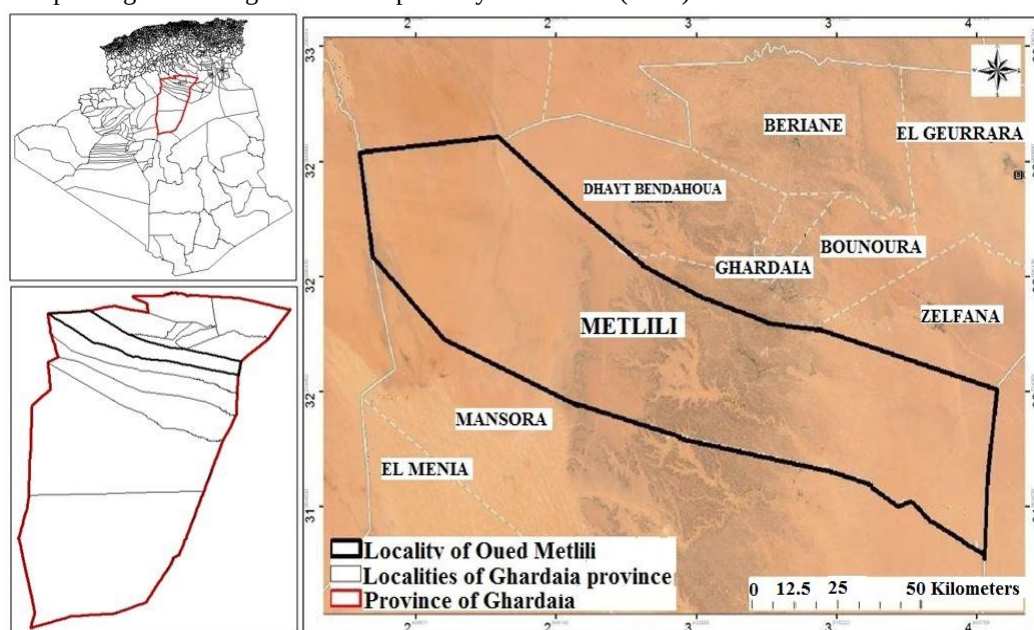
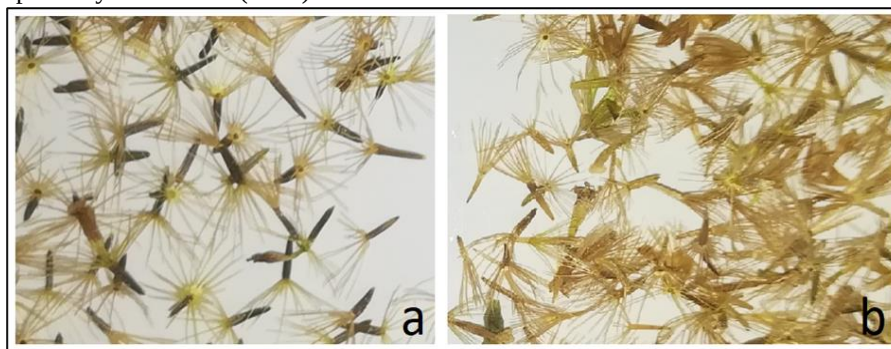


Figure 2. Selection Of *Stevia Rebaudiana* seeds From 2019, (a): Fertile Seeds, (b): Infertile Seeds. *Stevia rebaudiana* Bertonii is a plant of origin from the North-East of Paraguay, belonging to the family Asteraceae, subfamily Asteroideae, tribe Heliantheae, genus *Stevia*. Plants belonging to the genus *Stevia* are annual or perennial herbs or shrubs, primarily found between 500 and 3000 meters above sea level, usually in mountainous environments semi-dry. The unselected seeds were spread out on a previously cleaned and disinfected work surface. We delicately sorted the fertile seeds from the infertile ones using metal tweezers. Fertile seeds are usually dark in color, while infertile seeds are usually pale or light. Source: Prepared by the authors (2020).



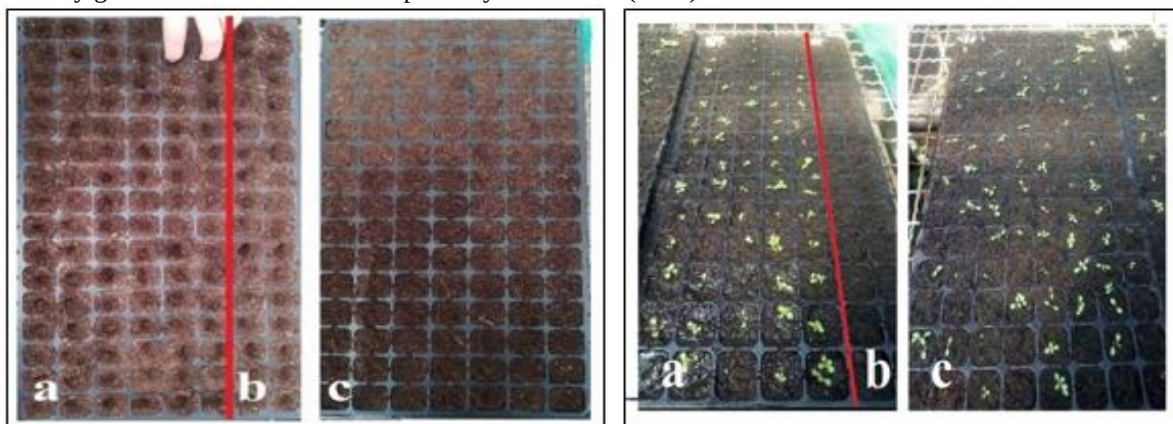
2.2.1 Germination

A specific temperature of 20°C to 25°C for the germination of *Stevia* seeds was ensured in the greenhouse of the higher school of agronomy in the province of Oran. The unselected seeds from the 2019 batch were spread out on a previously cleaned and disinfected work surface. We then gently sorted the fertile seeds from the infertile seeds using metal tweezers. Fertile seeds are usually dark in color, while infertile seeds are usually pale or light. The selected seeds retained for our experiment were disinfected in diluted sodium hypochlorite for 15 min. They are then rinsed 3 times with distilled water, then placed in Petri dishes, lined with two sheets of filter paper soaked in 4 ml of distilled water. We retained three (03) repetitions. The boxes are then left at a temperature of 25°C. Daily counts of the number of germinated seeds were carried out. The preliminary germination test allowed us to note that the germinated seeds had a germination rate greater than 50%.

2.2.2 Sprouted seeds

The germination trays mentioned are filled with “special seedling” soil. We opted for this commercial mixture based on good potting soil and river sand (2/3 potting soil for 1/3 sand). We ensured the following conditions: ambient temperature of 25 degrees, high humidity (relative humidity between 70 and 90%) and 10 hours of daily lighting. We proceeded as for any other plant, we filled the cells with a special “light” seeding mixture, then placed the selected seeds directly on the surface. To improve contact with the ground, it was lightly compacted and covered with a very thin layer. Then we started humidifying everything with a sprayer once a day.

Figure 3. Sowing *Stevia rebaudiana* seeds, three sowing trials of selected seeds from 2019, (a): semi with preliminary germination test, (b): semi without preliminary germination test of seeds aged three years and (c): semi without preliminary germination test. Source: Prepared by the authors (2020).



2.2.3 Transplantation

To transplant the plants, we used a balanced substrate, watering two to three times a week and an ambient temperature of 20°C during the day. However, our plant is very sensitive to direct exposure to sunlight as well as frost (low temperatures) during the night. In order to protect our young plants, a shelter system was created like a blanket to limit the cold and excess humidity.

2.3 PHYTOCHEMICAL ANALYSIS

2.3.1 Selection, drying and grinding

The Stevia leaves obtained after a few months were sorted and dried. In order to preserve them as long as possible by applying the drying method in a ventilated oven. The leaves of our plant were dried according to the procedure of (Ceccanti et al., 2021). Hot drying was carried out at 55°C for 48 hours. After drying, we noticed that the leaves are lighter than before, a sign that our leaves are very dry. The dried leaves were stored at room temperature, in tightly closed and labeled sterile glass jars until the day of extraction. For leaf crushing, we used a simple and easy method to crush the leaves: in a manual mortar and with a disinfected pestle, without the use of liquid nitrogen. (Mabosy et al., 2021).

2.3.2 antioxidant activity

The antioxidant activity of the purified ethanolic and aqueous extracts of Stevia rebaudiana as well as the standard antioxidant (ascorbic acid) against the DPPH radical were evaluated using a spectrophotometer. The reduction of this radical was followed by its transition from the violet color (DPPH) to the yellow color (DPPH-H) measurable at 517 nm (Tlili et al., 2020). This reduction capacity is determined by a decrease in absorbance induced by anti-radical substances which indicates the presence of antioxidant activity (Tenda et al., 2019).

2.3.3 Phytochemical screening

Stevia extract was subjected to various phytochemical tests to identify the main chemical groups present in our plant, using a conventional method based on coloring and precipitation reactions. Several types of reagents have been used to achieve this objective (Bounihi, 2016).

2.3.4 Cardiac glycoside test

2 ml of chloroform was added to 1 ml of extract, the appearance of a reddish color upon addition of H_2SO_4 indicates the presence of cardiac glycosides (El-Haoud et al., 2018).

2.3.5 Tannin test

To detect the presence of tannins, 1 ml of extract was mixed with 1 ml of water and 1 to 2 drops of FeCl_3 solution diluted to (1%) (El-Haoud et al., 2018). i) A dark green or blue-green appearance reveals the presence of tannins. ii) The dark green appearance reveals the presence of catechic tannins. iii) The blue-green appearance reveals the presence of Gallic tannins.

2.3.6 Flavonoid test

2 ml of extract were evaporated and the residue was taken up in 5 ml of hydrochloric acid diluted twice. There is a release of heat and a pink-orange or purplish tint when 2 to 3 magnesium shavings are added. The addition of 3 drops of isoamyl alcohol intensified the coloring, confirming the presence of flavonoids (El-Haoud et al., 2018).

2.3.7 Saponin test

In a series of 10 tubes numbered from 1 to 10. The latter was prepared by aqueous, hydroalcoholic decoction or infusion. The volume of each tube was adjusted to 10 ml with distilled water and then shaken longitudinally for 15 seconds. They were left to rest for 15 minutes before measuring the height of the foam produced (El-Haoud et al., 2018).

2.3.8 Mucilage test

1 ml of decoction was put in a test tube, then 5 ml of absolute alcohol was added. The presence of mucilage is indicated by the presence of a flaky precipitate after shaking (El-Haoud et al., 2018).

2.3.9 Alkaloid test

10 g of dry vegetable powder were placed in an Erlenmeyer flask with 50 ml of H_2SO_4 diluted 1/10 with distilled water. The mixture was stirred and soaked for 24 hours. Then, 5 drops of Dragendorff reagent were added to 1 ml of filtrate. The appearance of an orange precipitate indicates the presence of alkaloids (El-Haoud et al., 2018).

2.3.10 Testing essential oils

1 g of plant material was dissolved in 10 ml of dichloromethane then evaporated at low temperature. The resulting residue was then introduced into 3 ml of ethanol and the solution was again evaporated to dryness. The presence of essential oils is indicated by the sensation of a fragrant odor (El-Haoud et al., 2018).

2.3.11 Extraction of steviol glycosides

Extraction is a crucial step in the discovery of bioactive components in plants. Among these compounds are steviol glycosides which are secondary metabolites. The protocols developed focus on four extractions: infusion, decoction, maceration and finally by soxhlet.

2.3.12 Extraction by infusion

100 ml of hot distilled water (solvent) was added to 10 g of prepared plant material, which was kept boiling for 30 minutes at 70°C. After cooling, the resulting solution was filtered through Buchner and filter paper, and the resulting residue was refiltered 5 times until the plant material was completely exhausted. A volume of filtrate was evaporated on a rotary evaporator at 100°C (Haouari et al., 2018).

2.3.13 Extraction by decoction

100 g of crushed leaves were mixed with 1 liter of hot water, stirred at a temperature of 95°C for 4 hours, the solution obtained was then filtered through filter paper. This extraction process is repeated once with the resulting residue until all the plant material is consumed. The filtrate is concentrated under vacuum on the rotary evaporator with a vacuum pump below the evaporation point of the solvent 100°C (Haouari et al., 2018).

2.3.14 extraction by maceration

10 g of sample was macerated in 100 ml of hydroalcoholic solution (70% ethanol), for 48 hours at room temperature. Then the resulting solution was filtered. The latter was evaporated using the rotary evaporator at a temperature of 80°C to obtain the crude extract (Marsol-vall et al., 2022).

2.3.15 Extraction by soxhlet

7 g of powdered leaves were put in a Soxhlet cartridge. The extraction was carried out using a soxhlet assembly with a 250 ml flask which contained 200 ml of ethanol in a heating flask set at 40°C. The extraction operation lasted 4 days (7 hours each day) to exhaust the plant material. The resulting solution was vaporized by the rotary evaporator at 80°C to obtain the crude extract (Ribeiro et al., 2021).

2.3.16 Purification of glycosides

2.3.16.1 pH purification

The filtrate obtained by the infusion method contains several suspended particles. To remove them, we measured 1 g of calcium hydroxide Ca(OH)_2 and added it to the solution (extract). It

serves as a flocculant to help the filtrate form larger particles, which are then filtered. 1 g of citric acid commonly used in the food industry was added to reduce the pH of the solution to 7. A volume of 120 ml was evaporated to recover the yield.

2.3.16.2 purification by organic compounds

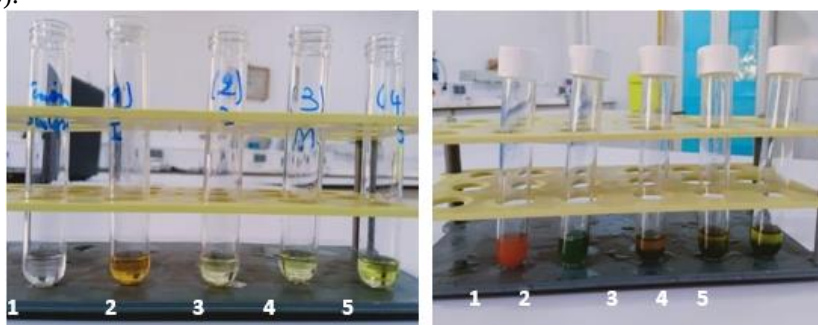
In this method, we use the extracts obtained by the four methods. Using a separatory funnel, the aqueous extracts were washed with ethyl acetate, dividing them into two phases (aqueous and organic). The aqueous phase was recovered and washed with n-butanol which also allows separation into two phases, but this time the organic phase which was recovered because it is rich in steviol glycoside.

2.4 ANTIBACTERIAL EFFECTS

2.4.1 Revitalization of strains

Steviol glycosides extracted from *Stevia rebaudiana* were tested on 5 pathogenic bacterial strains: *Pseudomonas Auriginosa* ATCC 27857, *Bacillus Cereus* ATCC 6633, *Staphylococcus aureus* ATCC 5362, *Escherichia coli* ATCC 25922, *Klebsiella Pneumoniae*. The microbial strains were reactivated in (6 ml) nutrient broth then incubated at 37°C for 20 hours. The turbidity of the nutrient broth indicates the development of the cultured strains and compared to the McFarland reference. The extracts were evaporated and then collected in distilled water for the antibacterial test. Flooding or sheet cultivation technique is applied for seeding. The entire surface of the solid medium (Mueller Hinton agar) is flooded with the inoculum to be tested, after adding 70 µl of the four extracts at a concentration of (2 mg/ml) to the wells. The plates are incubated in an incubator at 37°C for 21 hours (Nacer et al., 2012). After 24 hours of incubation, the presence of an inhibition zone was observed and the inhibition diameter was measured.

Figure 4. Fehling test of the steviol glycoside extracts, the five extracts obtained are: glucose, infusion extract, decoction extract, maceration extract, soxhlet extract. on the left the figure Before the test and on the right the figure after the test. the absence of color change and brick red precipitate in the five purified extracts. This is due to the absence of the aldehyde function which is oxidized by copper to give a carboxylic acid and a brick red precipitate of cuprous oxide. These results indicate that the steviol glycosides present in our samples would be non-reducing sugars. Source: Prepared by the authors (2020).



3 RESULTS AND DISCUSSION

3.1 GERMINATION TEST RESULTS

Germination of selected *Stevia rebaudiana* seeds began in the first week of sowing. During the six weeks following sowing of the seeds selected for 2019, the plants reached a length of 03 cm. The germination rate recorded in both types was above 50%. The transplanting of *Stevia rebaudiana* outside the greenhouse was successful because the new plants recorded a length reaching 40 cm in three (03) months. After three months, the average measurements of the different parts of the plant were presented in Table 1, Figures 4 and 5.

3.2 ANTIOXIDANT ACTIVITY RESULTS

The results of the antioxidant power of the extracts tested show that the percentage of inhibition of the DPPH radical as a function of the concentrations of the compounds tested increases with the concentrations, whether for ascorbic acid or the extracts. For each concentration tested, stevia extracts exhibit antioxidant activity against DPPH radicals. However, by comparing the four extracts from the different methods, we observed an inhibition percentage of between (35% and 99%) for the four extracts: infusion, maceration, Soxhlet and decoction. But the percentage of inhibition of the extract from the decoction is higher than that of the other three extracts. on the other hand, concerning the percentage of inhibition of ascorbic acid is greater than that of all the extracts for all concentrations; it is between (50% and 99%). The IC₅₀ of the three extracts are respectively (0.32 ± 0.009 mg/ml), (0.020 ± 0.0014 mg/ml) and (0.013 ± 0.008 mg/ml), are relatively high compared to that of ascorbic acid (0.0017 ± 0.0003 mg/ml).). However, it was found that the fourth value recorded from the decoction (0.006 ± 0.0001 mg/ml) is close to that of ascorbic acid. This indicates that the aqueous decoction extract has better antioxidant activity (Figure 6).

3.3 PHYTOCHEMICAL SCREENING RESULTS

The phytochemical tests illustrated in Table 2 and which are represented in Figure 7, highlight the presence of alkaloids, cardiac glycosides, catechic tannins, flavonoids, saponins, mucilages and essential oils.

3.4 STEVIOL GLYCOSIDE EXTRACTION RESULTS

Table 02 summarizes the yield results of the different extracts of the plant studied obtained by extraction of steviol glycosides according to four different methods, using ethanol or water as solvent, expressed as a percentage (%): relative to the dry weight vegetable powder. It is noted that the extract yields vary from one method to another. the decoction method gave the best yield with

a percentage of 17.6, followed by that of infusion with (12.6%) and that of Soxhlet (10.4%). We recorded the lowest rate with the maceration method (8.3%).

Table 1: Averages of the different parameters of *Stevia rebaudiana* measured

Height of whole plant	40 cm
Height of main stems	24,5 cm
Number of main (central) stems	1
Total weight of stems	5,8 g
Weight of the whole plant	7,6 g
Weight of green leaves (raw)	0,5 g
The weight of the roots	1,4 g

Figure 5. Evolution of *stevia* plants in a period of six weeks inside greenhouse, (a): *Stevia* seedlings with preliminary germination test; (b): Absence of germination of selected seeds aged three years; (c): *Stevia* seedlings without preliminary germination test (Photos Belhadj, 2020).

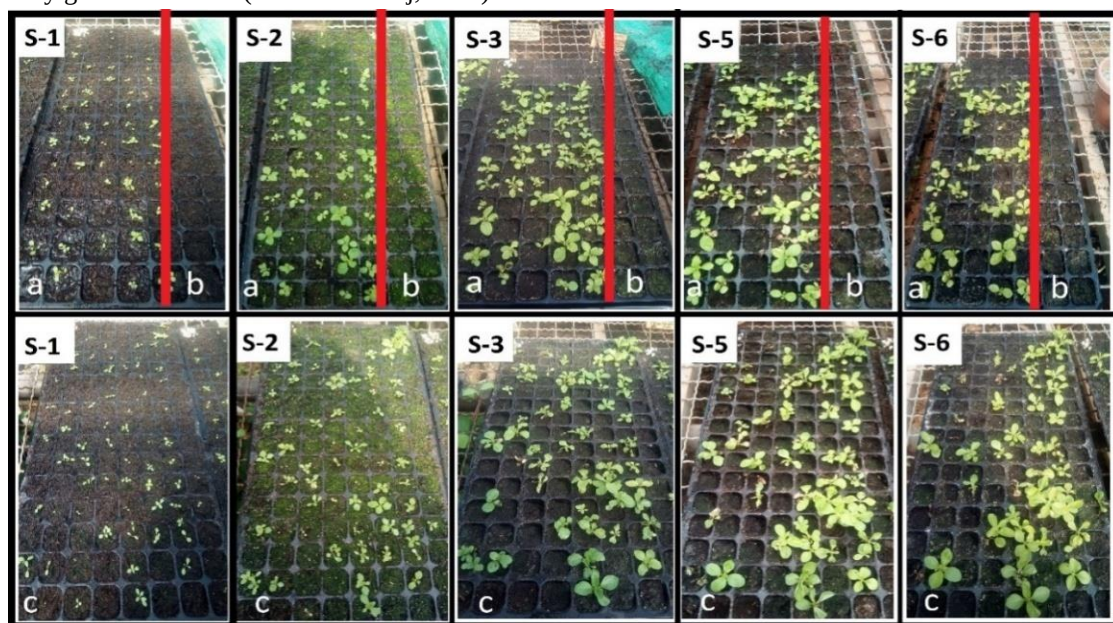


Figure 6. Results of transplanting seedlings after nine weeks (Photos Belhadj, 2020)



Figure 7. DPPH % inhibition curve for the different samples. Stevia extracts exhibit antioxidant activity against DPPH radicals. However, by comparing the four extracts from the different methods, we observed an inhibition percentage between (35% and 99%) for the four extracts: infusion, maceration, Soxhlet and decoction. But the percentage of inhibition of the extract from the decoction is higher.

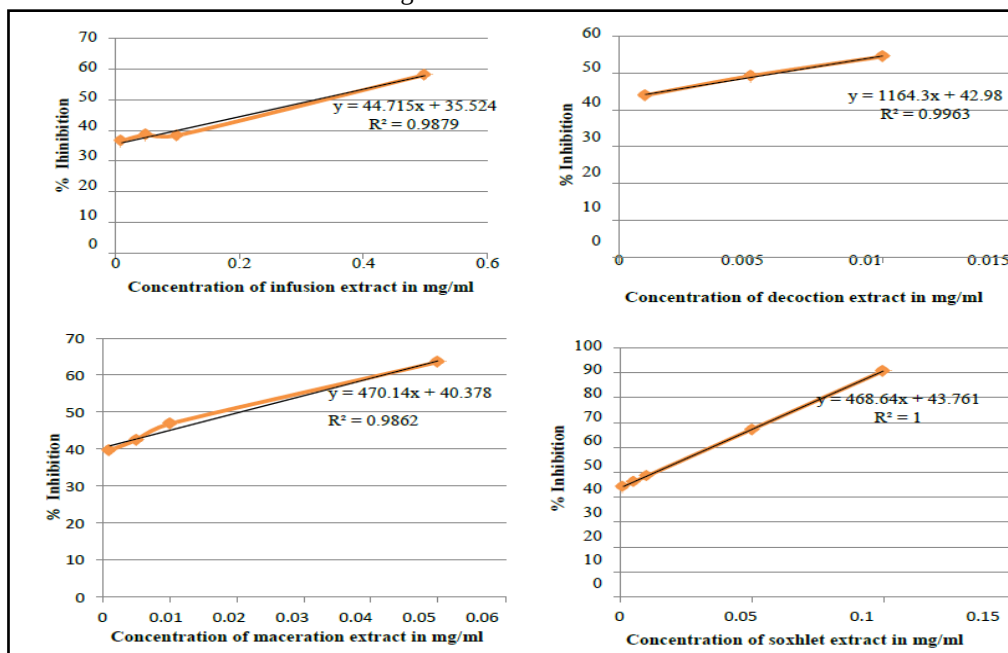


Figure 8. The IC₅₀ values of the different extracts. The IC₅₀ of the first three extracts are relatively high than that of ascorbic acid. However, the fourth value recorded from the decoction was found to be close to that of ascorbic acid. This indicates that the aqueous decoction extract has better antioxidant activity.

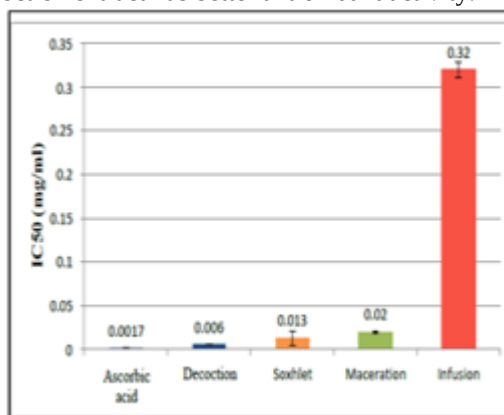
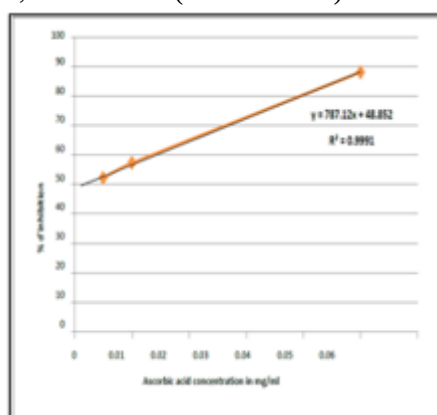


Figure 9. Curve of % inhibition of DPPH by ascorbic acid. The percentage of inhibition of ascorbic acid is higher than that of all extracts for all concentrations; it is between (50% and 99%)



3.5 ANTIBACTERIAL EFFECT RESULTS

The results of the antibacterial effect of the extracts are grouped in Table 04. The values indicated are the averages of three measurements. The bacteriostatic action results in the appearance of a zone of inhibition around the well of the extracts studied. The results obtained for the antibacterial activity of the decoction extract show that there is sensitivity on three strains tested (*B. cereus*, *S. aureus*, *P. auriginosa*) with an inhibition zone of (1.36 ± 0.05 cm), (1.43 ± 0.05 cm), (1.16 ± 0.05 cm) respectively. For the other two strains (*K. pneumoniae*, *E. coli*) no effect was observed on them. the maceration and Soxhlet extracts are active on two strains (*B. cereus*, *S. aureus*); the extract resulting from maceration with an inhibition zone respectively of (1.15 ± 0.07 cm), (1.2 ± 0.17 cm), that of Soxhlet is respectively (1.1 ± 0.1 cm), (1.06 ± 0.05 cm). These extracts are resistant to gram negative microbial strains. In fact, the extract of the infusion did not react with any strain.

Table 2: Results of phytochemical screening. Phytochemical screening made it possible to characterize some existing chemical groups in the crude aqueous extract of stevia leaves. the presence of alkaloids, cardiac glycosides, catechic tannins, flavonoids, saponins, mucilages and essential oils have been demonstrated

The compounds	Alkaloids	Cardiac Glycoside	Tannin	flavonoids	saponins	Mucilages	Essential oils
Appearance or color	orange	Reddish brown	Dark green	Orange-pink	I=166	Appearance of foam	Fragrant smell
Expression of results	+	+++	+++	+	+++	+++	+

Table 3: Antimicrobial activity of steviol glycoside extracts from *S. rebaudiana*. the steviol glycoside from the leaves of *Stevia rebaudiana* has inhibitory activities on the growth of most pathogenic microorganisms and the quantity of steviol glycosides controls the difference in the inhibitory activity between the extracts. the decoction extract implies higher inhibitory activity on *B. cereus*, *S. aureus*, *P. auriginosa*. On the other hand, the two ethanolic extracts have low inhibitory activity. On the same strains the infusion extract does not exert an inhibition.

Strains Bacterial	Gram +		Gram -		
	<i>Bacillus cereus</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas auriginosa</i>	<i>Klebsiella pneumoniae</i>	<i>Escherichia coli</i>
Decoction extract	1.36 ± 0.05	1.43 ± 0.05	1.16 ± 0.05	-	-
Maceration extract	1.15 ± 0.07	1.2 ± 0.17	-	-	-
Excerpt from Soxhlet	1.1 ± 0.1	1.06 ± 0.05	-	-	-
Infusion extract	-	-	-	-	-

Optimization of the germination of *stevia rebaudiana* in the Oran province shows that the seeds are not dormant and that they are of good quality. Its seeds are capable of germinating easily in favorable conditions of humidity and temperature. Several authors report that complex and diverse factors are responsible for seed dormancy and are associated with changes in the pericarp, seed coat and embryo when viable seeds fail to germinate or germinate with difficulty in a usually

suitable environment (Costa, 2020; Mooradian, 2019; Goyal et al., 2010). The germination of the selected Stevia seeds began in the first week after sowing the seeds, with temperatures varying between 22°C and 28°C and a light intensity of 13 hours. Temperature plays an important role in seed germination of species and each plant species has distinct temperature requirements for germination (Imran et al., 2022). The optimal temperature for seed germination depends on the nature of the crop and the environmental conditions faced by the maternal plants (Petit et al., 2020). The optimal germination temperature of Stevia seeds has been reported to be 25°C in previous studies (Imran et al., 2022; Petit et al., 2019; Petit et al., 2020). Some previous work has been done on optimizing Stevia seed germination at an optimal temperature of 25°C (Libik-Konieczny et al., 2019, Libik-Konieczny et al., 2018, Lucho et al., 2019, Lucho et al., 2018). Similarly, light has been reported to have a positive influence on seed germination (Ng et al., 2019, Imran et al., 2022). It is recommended to ensure a lighting duration of 14 to 16 hours per day to increase the germination rate (Petit, 2019) and its cultivation in a temperate environment would allow foliar production and therefore higher steviol glycoside (Morlock et al., 2002; Ng et al., 2019). The substrates in which the seeds are sown also influence their germination and shoot development. A light, clay-sandy and limestone soil would be the best substrate, while a heavy, clayey soil considerably affects the good growth of stevia plants (Barbet-Massin, 2015). The transplanting was carried out at the right time and in conditions favorable to the growth of the plants, from which the latter were able to reach (40 cm) height in three (3) months, showing good signs of vitality and health, and no illnesses were observed. In Morocco, in many provinces where Stevia is cultivated, whose climate is similar to that of the Oran province; one study showed that the height of the plant can reach up to 70 (Cm) (Barbet-Massin, 2015). In its original habitat, based on our illustrated experience, we will estimate that in three (3) months our plants will reach this height. Another study was carried out in Central Bulgaria, on the cultivation of the natural sweetener Stevia rebaudiana, where the height of the plant reached up to (40 to 60 cm) in a period of four (04) months (Libik- Konieczny et al., 2018). The results of the phytochemical screening made it possible to characterize certain chemical groups existing in the crude aqueous extract of stevia leaves. Phytochemical tests highlight the presence of alkaloids, cardiac glycosides, catechin tannins, flavonoids, saponins, mucilages and essential oils. These results are in agreement with the work carried out by Soufi et al., 2015; Blinstrubienè et al., 2020; Turko et al., 2007. According to Vidyanagar (2006), Stevia extracts contain only tannins, alkaloids, cardiac glycosides and saponins. Siddique et al.; 2014; also claims the presence of tannins, alkaloids, flavonoids and saponins. Kharchouf et al., (2017); showed the absence of alkaloids in the leaves of Stevia, cultivated in Morocco. This is due to the difference in substrates and climatic conditions which can affect the chemical composition of this plant (Woelwer et al.,

2010). Libik-Konieczny et al., (2018); show that high levels of phenolic compounds vary independently depending on cultivation location, difference in morphology and photosynthetic efficiency. Different parameters control the performance of steviol glycosides, such as: solvent, temperature, contact time between the solvent and the steviol glycoside (Ursula, 2018; Jentzer et al., 2015). Our results show that the yield of steviol glycosides from the aqueous extract of the decoction and the infusion is higher than the yield of the ethanolic extracts from maceration and Soxhlet. This indicates that steviol glycoside might favor solubility in water rather than ethanol, since it seems logical that polar solvents would be preferred over nonpolar solvents, since glycosides are polar molecules. These results are in agreement with the work of Sandra et al., 2020; Ribeiro et al., 2021; which demonstrate that ethanol slightly reduces the total glycoside content compared to water (Espinoza et al., 2022). Other works by Abou-Arab et al., (2010); notes that water also gives a good yield than methanol. In other words, water was found to be the best solvent than all organic solvents for the recovery of steviol glycosides. According to Rai et al., 2012; Kovačević et al., 2018; temperature plays a major role in the extraction process of steviol glycosides, they vary in temperature (60 to 100°C) and contact time (4 to 24 hours). Although steviol glycosides are thermostable and relatively polar compounds (Lemus et al., 2012; Erkucuk et al., 2009), when the temperature increases up to (150°C), the yields decrease significantly. This would be due to the fact that at moderate temperature and medium contact time, water would solubilize polar compounds more and at high temperatures, it would solubilize non-polar compounds more (Yildiz-Ozturk et al., 2014). Indeed the study carried out by Yang et al., 2019; reported that the yield of steviol glycosides from new extraction methods is approximately (68-75%), the latter being alternatives to conventional methods to improve the production of high purity steviol glycosides, which is also in agreement with Castro et al., 2021. The results of the antibacterial effect obtained allowed us to know that the steviol glycoside of *Stevia* leaves has inhibitory activities on the growth of most pathogenic microorganisms and that the amount of steviol glycosides controls the difference in inhibitory activity between the four extracts, but their mechanism is unknown. The same remark was mentioned by Khatun et al., 2021 ; Deonas et al., 2022. This type of inhibition has already been detected on the same microorganisms with Rakesh et al., 2013 and Espinoza et al., 2022. On the other hand, we recorded a slight inhibitory effect of glycosides of steviol on *E. coli* and *P. Auriginosa*.

4 CONCLUSION

Stevia, over time, continues to revive the market for sweeteners, which are still considered products with a high chemical content. This state continues to push potential consumers to lean

more and more towards natural products, little transformed. Consequently, the plant gradually invades our daily diet and fits into it perfectly. Also and for the moment it presents the most advantages: calorie-free, non-Cariogenic and of natural origin. Our work was essentially based on the optimization of the cultivation of the *Stevia rebaudiana* plant in the Oran province Northwestern Algeria. This approach revealed to us that the germination of stevia seeds originating from the Ghardaïa Oasis was successful, as was the transplanting of young seedlings outside. Which shows that our plant has found a good adaptation; to the Mediterranean climate, humidity, soil quality, irrigation rate and temperature. It should also be noted that the plant was able to reach a height of (40 cm) after three months and that no subsidiary disease was observed on the new plants. Nevertheless and chronologically, it was estimated that the height of our plant could eventually reach a considerable height of (80cm to 100cm), as well as an appearance of the first flower buds. This will allow us to carry out a substantial and complete harvest with optimal yield. To this end, we were interested in the extraction of steviol glycosides from the leaves of the harvested *Stevia rebaudiana*. The extraction was carried out using four different methods and using different solvents. It is the decoction method which gave a better result to the water solvent which turned out to have a great affinity with the steviol glycoside also with the favorable operating conditions. According to the phytochemical screening, alkaloids and flavonoids, tannins, cardiac glycosides, saponins, mucilage and essential oils are present and the absence of reducing sugars in the extract of our stevia. The analysis of the antioxidant power shows that the steviol glycosides of this species have a significant antioxidant effect. The decoction gave us the best antioxidant activity which depends on the yield of steviol glycosides. The antimicrobial activity showed that the pathogenic bacteria *P. auriginosa*, *B. cereus* and *S. aureus* are sensitive to the different extracts but *E. coli* and *K. Pneumoniae* are resistant to these extracts. However, the decoction extract has the best inhibitory activity on all three bacteria as: *P. auriginosa*, *B. cereus* and *S. aureus*. Finally, our work opens the door to future work for the commercialization of steviol glycosides. This product with multiple virtues contains different phytochemical groups. This shows that it has an interesting future in the agri-food, pharmaceutical and even cosmetic fields.

ACKNOWLEDGEMENT

This study was carried out at the Higher School of Biological Sciences of Oran. We would also like to warmly thank the farmer Mr. Dahmane Abdelkader in the locality of Oued Metlili (province of Ghardaïa). Thanks to the team Toxicology, Environment and Health Laboratory, Faculty of Natural and Life Sciences, University of Science and Technology of Oran and the Algerian Directorate General of Scientific Research (DGRSDT) for their support for the success of this project.

REFERENCES

- Abou-Arab A.E, Abou-Arab A.A, Abu-Salem M.F., 2010. Bilan physico-chimique des édulcorants naturels stéviolides produits à partir de la plante *Stevia rebaudiana* Bertoni. *Journal africain des sciences alimentaires* , vol4 (5), 269-281.
- Arabi M., Makhlouf L., Sedji A., Bague A., Benlabiod D., Hamoudi A., Bensaada-hamouche F., 2021. Aménagements antiérosifs susceptibles de réduire l'intensité des crues dans le bassin versant de Ghardaïa en Algérie. *Annales de la Recherche Forestière en Algérie*, 11(2), 16-30.
- Barbet-Massin C., 2015. Sélectionner et cultiver *Stevia rebaudiana* Bertoni en milieu tempéré : exploration de la variabilité de la teneur et de la composition en glycosides de steviol. *Sciences agricoles*. Institut National Polytechnique de Toulouse - INPT. Français. ffnNT : 2015INPT0060ff. fftel04235160f .
- Blinstrubiene A., Burbulis N., Juškevičiūtė N., Vaitkevičienė N., Žūkienė R., 2020. Effect of growth regulators on *Stevia rebaudiana* Bertoni callus genesis and influence of auxin and proline to steviol glycosides, phenols, flavonoids accumulation, and antioxidant activity in vitro. *Molecules*, vol25(12), 2759.
- Borgo J., Laurella L.C., Martini F., Catalán C.A.N., Sülsen V.P., 2021. *Stevia* Genus: Phytochemistry and Biological Activities Update. *Molecules* Mdpi Vol26,2733.
- Bounihi A., 2016. Criblage phytochimique, Étude Toxicologique et Valorisation Pharmacologique de *Melissa officinalis* et de *Mentha rotundifolia* (Lamiacées) (Doctoral dissertation). Rabat institut, thèse de doctorat 208pp.
- Deonas A.N., Zorzenon M.R.T., Oliveira J.P. de, Silva D.V. da, Cabeça C.L.S., Mori, M.A., Costa, S.C. da, Milani, P.G., & França, E.J.G. de., 2022. *Stevia Rebaudiana* fraction presents broad-spectrum antibacterial action and antibiofilm action for *Staphylococcus aureus* / Fração *Stevia Rebaudiana* apresenta ação antibacteriana de amplo espectro e ação antibiótica para *Staphylococcus aureus*. *Brazilian Journal of Development*, 8(7), 49529–49541pp.
- Espinoza E.F., Villamarin B.J., Suarez G.L., Vásconez G.G., 2022. Acceptability of an infusion of Chamomile (*Chamaemelum nobile*) with stevia (*Stevia rebaudiana bertonii*) with health benefits, as an economic alternative for small entrepreneurs in the city of Babahoyo. *Brazilian Journal of Animal and Environmental Research*, Curitiba, v.5, n.2. 2163-2171pp.
- Castro-Muñoz R., Díaz-Montes E., Cassano A., Gontarek E., 2021. Membrane separation processes for the extraction and purification of steviol glycosides: an overview. *Critical reviews in food science and nutrition*, vol 61(13), 2152/2174.
- Castro-Muñoz R., Correa-Delgado M., Córdova Almeida R., Lara-Nava D., Chávez-Muñ M., Velásquez-Chávez V.F., Hernández-Torres D.E., Gontarek-Castro E., Zamidi Ahmad M., 2022. Natural sweeteners: Sources, extraction and current uses in foods and food industries, *Food Chemistry* Vol 15(370)130991.
- Ceccanti C., Finimundy T.C., Heleno S.A., Pires T.C., Calhella R.C., Guidi L., Barros L., 2021. Differences in the phenolic composition and nutraceutical properties of freeze dried and oven-dried wild and domesticated samples of *Sanguisorba minor* Scop. *LWT*, 145, /111335.

Chen Z., Shen Z., Hua Z., Li X., 2023. Global development and future trends of artificial sweetener research based on bibliometrics, , Ecotoxicology and Environmental, Safety, Vol263,115221.

Costa J.R., Tonon R.V., Cabral L., Gottschalk L., Pastrana L., Pintado M.E., 2020. Valorization of agricultural lignocellulosic plant byproducts through enzymatic and enzyme- assisted extraction of high-value-added compounds: A Review. ACS Sustainable Chemistry & Engineering, vol8(35),13112/13125.

Deliza R., Lima F.M., Ares G., 2021. Rethinking sugar reduction in processed foods, Current Opinion in Food Science elsevier Vol 40(4), 58-66pp.

Edwards C.H., Rossi M., Corpe C.P., Butterworth P.J., Ellis P.R., 2016. The role of sugars and sweeteners in food, diet and health: Alternatives for the future Trends in Food Science & Technology Vol56, 158-166pp.

EL-Haoud H., Boufellous M., Berrani A., Tazougart H., Bengueddour R., 2018. Screening phytochimique d'une plante medicinale: Mentha Spicata L. Journal of Innovative Research and Applied Sciences. 2429-5396.

Erkucuk A., Akgunlh Yesil-Celiktas O., 2009. Supercritical CO₂ extraction of glycosides from Stevia rebaudiana leaves : identification and optimization. J. Supercritical, Fluids, vol51,29-35.

Goyal S.K., Samsher Goyal R.K., 2010. Stevia (*Stevia rebaudiana*) un bio-édulcorant : un bilan. Revue internationale des sciences alimentaires et de la nutrition, 61(1), 1-10.

Haouari E., Makaou S.E., Jnah M., Haddaouy A., 2018. A survey of medicinal plants used by herbalists in Taza (Northern Morocco) to manage various ailments. *Journal of Materials and Environmental Science*, vol9, 1875-1888.

Hossain F., Islam M.T., Islam M.A., Akhta S., 2017. Cultivation and uses of Stevia (*Stevia Rebaudiana* Bertoni): A review African Journal of Food, Agriculture, Nutrition and Development Vol17(04):12745-12757.

Imran H., Rehman H., 2022. The impact of different seed priming agents and priming durations on stand establishment and biochemical attributes of *Stevia rebaudiana* Bertoni, Saudi Journal of Biological Sciences Vol29 (4), 2210-2218pp.

Jentzer J.B., Alignan M., Vaca-Garcia C., Rigal L., Vilarem G., 2015. Response surface methodology to optimise Accelerated Solvent Extraction of steviol glycosides from *Stevia rebaudiana* Bertoni leaves. *Food chemistry*, vol166, 561-567.

Kacper W., Karolina W., Małgorzata F., 2022. A High-Sugar Diet Consumption, Metabolism and Health Impacts with a Focus on the Development of Substance Use Disorder: A Narrative Review, *Nutrients* Mdpi , vol 14, 2940.

Khaled C., Lucette M., 2020. Piégeage Et Isolement Des Bactéries Nodulantes Des Légumineuses (Bnl) Présentes Au Niveau Des Rhizosphères De Quelques Espèces De Fabacées Spontanées Du Sahara Septentrional Algérien. *Algerian Journal of Arid Environment "AJAE"*, Vol10(1).

Kharchouf S., Bouchador A., Drioiche A., Khiya Z., El Hilali F., Zair T., 2017. Phytochemistry and antioxydante activity of *Stevia rebaudiana*. *Phytothérapie*, 1-7pp.

Khatun M.C.S., Muhit M.A., Hossain M.J., Al-Mansur M.A., Rahman S.A., 2021. Isolation of phytochemical constituents from *Stevia rebaudiana* (Bert.) and evaluation of their anticancer, antimicrobial and antioxidant properties via in vitro and in silico approaches. *Heliyon*, vol7(12), e08475.

Lamri L., Gripiotis E., Ferrario A., 2014. Diabetes in Algeria and challenges for health policy: a literature review of prevalence, cost, management and outcomes of diabetes and its complications. *Globalization and Health*. Vol24(10)11.

Lemus-Mondaca R., Vega-Gálvez A., Zura-Bravo L., Ah-Hen K., 2012. *Stevia rebaudiana* Bertoni, source of a high-potency natural sweetener: A comprehensive review on the biochemical, nutritional and functional aspects. *Food chemistry*, vol132(3), 1121-1132.

Leth T., Jensen U., Fagt S., Andersen R., 2005. Estimated intake of intense sweeteners from non-alcoholic beverages in Denmark. *Food Additives and Contaminants*, 25 (06), 662- 668pp.

Libik-Konieczny M., Capecka E., Kąkol E., Dziurka M., Grabowska-Joachimik A., Sliwinska E., Pistelli L., 2018. Growth, development and steviol glycosides content in the relation to the photosynthetic activity of several *Stevia rebaudiana* Bertoni strains cultivated under temperate climate conditions. *Scientia horticulturae*, vol234, 10-18.

Libik-Konieczny M., Capecka E., Tuleja M., Konieczny R., 2021. Synthesis and production of steviol glycosides: recent research trends and perspectives. *Applied Microbiology and Biotechnology*, vol 105(10), 3883-3900.

Liu Z., Wan X., 2020. A review: Natural polysaccharides from medicinal plants and microorganisms and their anti-herpetic mechanism, in *Biomedicine & Pharmacotherapy*, Vol 129, 110469.

Lucho S.R., do Amaral M.N., Auler P.A., Bianchi V.J., Ferrer M.A., Calderón A.A., Braga E.J.B., 2019. Salt stress-induced changes in vitro cultured *Stevia rebaudiana* Bertoni: effect on metabolite contents, antioxidant capacity and expression of steviol glycosides-related biosynthetic genes. *Journal of Plant Growth Regulation*, Vol38(4), 1341- 1353.

Lucho S.R., Do Amaral M.N., Milech C., Ferrer M.A., Calderón A.A., Bianchi V.J.,Braga E.J.B., 2018. Elicitor-induced transcriptional changes of genes of the steviol glycoside biosynthesis pathway in *Stevia rebaudiana* Bertoni. *Journal of Plant Growth Regulation*, Vol37(3), 971-985.

Mabossy-mobouna G., Malaisse F., 2021. Etude socio-économique de la consommation des feuilles de manioc (*Manihot esculenta* Crantz) dans la ville de Brazzaville (République du Congo). *Geo-Eco-Trop*, Vol45(1),9.

Margaret A., 2015. *Stevia*, Nature's Zero-Calorie Sustainable Sweetener A New Player in the Fight Against Obesity ?*Food and Nutrition*, Vol50(3).

Marsol-Vall A., Ainsa S., Lopez R., Ferreira V., 2022. Development and validation of a method for the analysis of halophenols and haloanisoles in cork bark macerates by stir bar sorptive extraction heart-cutting two-dimensional gas chromatography negative chemical ionization mass spectrometry. *Journal of ChromatographyA*, Vol1673, 463186.

Mooradian A.D., 2019. In search for an alternative to sugar to reduce obesity. *International Journal for Vitamin and Nutrition Research*, Vol89(3-4), 113-117.

Morlock G.E., Heil J., 2020. HI-HPTLC-UV/Vis/FLD-HESI-HRMS and bioprofiling of steviol glycosides, steviol, and isosteviol in Stevia leaves and foods. *Analytical and bioanalytical chemistry*, vol412(24), 6431-6448.

Narayan K.M.V., Zhang P., Kanaya A.M., Williams D.E., Engelgau M.M., Imperatore G., Ramachandran A., 2011. Diabetes: The Pandemic and Potential Solutions, 2nd edition Review from The International Bank for Reconstruction and Development /The World Bank, Washington (DC), 21 Jan 2011PMID: 2125035.

Nazrul I.M.d., Bony A.M.D., Kwashie M.F., Ashiqur R.M.D., Raisul H.M.D., Aktarujjaman M.D., Farjana N., Roy N., 2020. Food safety and handling knowledge and practices among university students of Bangladesh: A cross-sectional study, Hileyon, Vol8(12), e11987.

Ng A.W., Loh K.K., Gupta N., Narayanan K., 2019. A polyol-stevia blended sugar replacer exhibits low glycemic response among human subjects. *Clinical nutrition ESPEN*, vol33, 39-41.

Pang M.D., Goossens G.H., Blaak E.E., 2021. The Impact of Artificial Sweeteners on Body Weight Control and Glucose Homeostasis *frontiers in nutrition* Vol (7).

Petit E., Jacques A., Daydé J., Vallejo V., Berger M., 2019. UGT76G1 polymorphism in Stevia rebaudiana: New variants for steviol glycosides conjugation. *Plant Physiology and Biochemistry*, vol135, 563-569.

Petit E., Berger M., Camborde L., Vallejo V., Daydé J., Jacques A., 2020. Development of screening methods for functional characterization of UGTs from Stevia rebaudiana. *Scientific reports*, vol10(1), 1-10.

Purkayastha S., Kwok D., 2020. Metabolic fate in adult and pediatric population of steviol glycosides produced from stevia leaf extract by different production technologies. *Regulatory Toxicology and Pharmacology*, vol116, 104727.

Rai C, Majumdar G.C., De S., 2012. Optimization of process parameters for water extraction of stevioside using Response Surface Methodology. *Separation Science and Technology*, vol47, 1014-1022.

Ramesh K., Singh V., Megeji N.W., Ramos-Tovar E., Muriel P., 2006. Cultivation of Stevia, Stevia rebaudiana (Bert.) Berton: A Comprehensive Review *Advances in Agronomy* vol89:137-177pp.

Ramos-Tovar E., Muriel P., 2019. Dietary Interventions in Liver Disease *Foods, Nutrients, and Dietary Supplements Chapter 9 - Phytotherapy for the Liver*, 101-121pp.

Ribeiro M.M., Diamantino T., Domingues J., Montanari Í., Alves M.N., Gonçalves J.C., 2021. Stevia rebaudiana germplasm characterization using microsatellite markers and steviol glycosides quantification by HPLC. *Molecular biology reports*, vol48(3), 2573-2582.

Ribeiro M. M., Diamantino T., Domingues J., Montanari Í., Alves M.N., Gonçalves J.C., 2021. Stevia rebaudiana germplasm characterization using microsatellite markers and steviol glycosides

quantification by HPLC. *Molecular biology reports*, vol 48(3), 2573-2582.

Sandra P., Zorić Z., Bursać Kovačević D., Elez Garofulić I., Dragović-Uzelac V., 2020. Extraction à l'eau chaude sous pression des composés phénoliques des feuilles de *Stevia rebaudiana* : une étude UPLC-ESI-MSMS. *Journal of Food Process Engineering* , Vol43 (2), e13319.

Saraiva A., Carrascosa C., Raheem D., Ramos F., Raposo A., 2020. Natural Sweeteners: The Relevance of Food Naturalness for Consumers, Food Security Aspects, Sustainability and Health Impacts, *Int. J. Environ. Res. Public Health*, Vol17(17).

Siddique A.B., Rahman S.M.M., Hossain M.A., Rashid M.A., 2014. Screening phytochimique et potentiel antimicrobien comparatif de différents extraits de feuilles de *Stevia rebaudiana* Bertoni. *Journal Asie-Pacifique des maladies tropicales* , 4 (4), 275-280.

Soufi S., Bettaieb T., 2015. Notes ethnobotanique et phytopharmacologique sur *Stevia rebaudiana* Bert. *Phytothérapie*, vol13(3), 202-207.

Tenda P., Hilaria M., Wijaya H., 2019. Antioxidant Activities and Quality Test of Instant Faloak (*Sterculia quadrifida* R.Br.) From East Nusa Tenggara With Added Ginger (*Zingibera officinale* Roch). *International Journal of Advancement in Life Sciences Research*. Vol 2(4) 15-20.

Tlili M.L., Hammoudi R., Dehak K., Mahfoud H.M., 2020. Etude de l'activité antioxydants et antimicrobienne des extraits de feuilles de *pergulariatomentosa* issue d'el oued (ALGERIE). *Revue des bio-ressources*, Vol 10(2), 11-11.

Turko Y.A., Korobko N.V., Shokun V.V., Chernyak E.N., Vyalkov A.I., Stepankina O.N., Kerimzhanova B.F., Baltaev U.A., 2007. GC—MS research. I. Essential oil from *Stevia rebaudiana* *Stevia rebaudiana*. *Chemistry of Natural Compounds* 43 (6): 744–745.

Vallejo V.A., Warner R.M., 2021. Identifying quantitative trait loci for steviol glycoside production in *Stevia rebaudiana* using transcriptome-derived SSRs. *Industrial Crops and Products*, vol161, 113176.

Vidyanagar V., 2006. Preliminary studies on *Stevia rebaudiana* leaves: proximal composition, mineral analysis and phytochemical screening. *J. Med. Sci*, Vol6(3), 321-326.

Wang D.D., Li Y., Afshin A., Springmann M., Mozaffarian D., Stampfer M.J., Hu F.B., Murray C.J.L., Willett W.C., 2019. Global Improvement in Dietary Quality Could Lead to Substantial Reduction in Premature Death. *The Journal of Nutrition Community and International Nutrition*;Vol149(6),1065-1074pp.

Waqas S., Tahir A., Nadeem Shafique B., Mohd Rashid A.H., 2017. Effect of diet on type 2 diabetes mellitus: A review. *International Journal of Health Sciences* Vol. 11(2).

Wilk K., Korytek W., Pelczyńska M., Moszak M., Bogdański P., 2022. The Effect of Artificial Sweeteners Use on Sweet Taste Perception and Weight Loss Efficacy: A Review , *Nutrients*, vol14, 1261pp.

Woelwer-Rieck U., Lankes C., Wawrzun A., Wüst M., 2010. Improved HPLC Improved HPLC method for the evaluation of the major steviol glycosides in leaves of *Stevia rebaudiana* *European Food Research and Technology* vol231(4):581-588.

World Health Organization. Countries: Algeria. 2012.

Yadav A.K., Singh S., Dhyan D., Auhja P.S., 2011. A review on the improvement of stevia, *Stevia rebaudiana* (Bertoni), Canadian Journal of Plant Science Vol 91(1).