Antioxidant activity, phenol and flavonoid content, and formulation cream of *Stevia rebaudiana* Bert

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Abstract. The presented study aimed to characterize the content, biological activity and formulation of extracts prepared from dried *Stevia rebaudiana* Bert leaves. *Stevia rebaudiana* Bert is the leaves that have antioxidant activity. The leaves, stems and flowers of stevia contain a complex mixture of sweet diterpene glycosides, including isosteviol, stevioside, rebaudioside (A, B, C, D, E, and F), steviolbioside and dulcoside A. In addition to glycosides, the leaves of stevia also contain other phytoconstituents, such as flavonoids, phenolic acids, fatty acids, proteins, and vitamins. These compounds can prevent and retain free radicals substitution, which can cause aging and chronic diseases. The extract obtained was filtered and evaporated — determination of total phenolics and total flavonoid using spectrophotometric methods. The antioxidant potential of this extract was evaluated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. In the DPPH radical-scavenging activities, the extract had the antioxidant activity (IC₅₀ = 48.18 µg/ml). The results showed that these extracts of *Stevia rebaudiana* Bert could be considered as natural antioxidants. The evaluation of the formulated cream showed good results and can be good potential for cosmetic product development.

1. Introduction
Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer, and AIDS [1]. The antioxidant activity of plants is most often due to the high content of phenolic acids (gallic, protocatechuic, caffeic, and rosmarinic acids), phenolic diterpenes (carnosol, carnosic acid, rosmarinol, and rosmadial), flavonoids (quercetin, catechin, naringenin, and kaempferol), and volatile oils (eugenol, carvacrol, thymol, and menthol) [2].

*Stevia rebaudiana* Bert is the leaves that have antioxidant activity. The leaves, but also stems and flowers of stevia contain a complex mixture of sweet diterpene glycosides, including isosteviol, stevioside, rebaudioside (A, B, C, D, E and F), steviolbioside and dulcoside A. In addition to glycosides, the leaves of stevia contain other phytoconstituents, such as flavonoids, phenolic acids, fatty acids, proteins and vitamins [3, 4]. These compounds can prevent and retain free radicals substitution, which can cause aging and chronic diseases. The content of a broad range of biologically active substances makes *Stevia rebaudiana* Bert a valuable ingredient not only for food products but also for cosmetics.
This study aims to develop products with herbal antioxidant extracts as a way to protect the skin [5]. 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) is a fast, simple and inexpensive way to determine the antioxidant capacity of a material that has been widely used in testing because it can act as a hydrogen donor and evaluate antioxidant activity [6]. DPPH mechanism is based on a reduction in DPPH and stable free radicals [7].

2. Materials and methods
2.1. Materials
2,2-Diphenyl-1-picrylhydrazyl (DPPH). Stevia rebaudiana Bert leaves from Solo. Grade for the materials used is all analytical.

2.2. Extraction method
The Stevia rebaudiana Bert leaves were macerated with ethanol. The solution was filtered and concentrated under reduced pressure at 50°C.

2.3. Phenol and Total Flavonoid Content
The present study was carried out to evaluate the content of total phenols and flavonoids in extracts prepared from dried Stevia rebaudiana Bert leaves. Total flavonoids determined by each plant extracts (0.5 ml) in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured with a spectrophotometer. Total phenol values are expressed regarding gallic acid equivalent (mg g⁻¹ of dry mass), which is a common reference compound.

2.4. Antioxidant assay by the use of dpph free radical method
The stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) was used for the determination of free radical-scavenging activity of the extracts [9]. Different concentrations of each herbal extract were added, at an equal volume, to a methanolic solution of DPPH. After 15 min at room temperature, the absorbance was recorded at 517 nm. Ascorbic acid was used as standard controls. IC50 values denote the concentration of the sample, which is required to scavenge 50% of DPPH free radicals. This activity is given as the percent of DPPH radical scavenged, which is calculated with the equation: DPPH radical scavenging activity (%) = [(Abs control – Abs sample)/(Abs control)] × 100%

2.5. Preparation of Formulation
Stevia rebaudiana Bert extract is used to prepare the cream. The composition of cream can be seen in table 1. the oily phase of stearic acid and glycerin is melted at a temperature of 70°C. Aquos phase of sodium tetraborate and TEA is added. After that, extracts and methylparaben were added and prepared with aquadestillata.

| Table 1. The composition of cream Stevia rebaudiana Bert extract |
|---------------------------------------------------------------|
| **Components**                        | **Amount** |
| Stevia rebaudiana Bert extract         | 5%         |
| Stearic acid                           | 142        |
| Glycerin                               | 100        |
| Na. Tetraborate                        | 2.5        |
| TEA                                    | 10         |
| Methylparaben                          | 0.1        |
| Distilled water                        | 750        |
2.6. Evaluation of Antioxidant Cream

2.6.1. Physical Properties
The physical properties of the cream are observed in shape, color, odor, and PH.

2.6.2. Determination of Emulsion Type (Dye test)
The emulsion type was determined by methylene blue dye test. Drop of cream on a glass object and examined under a microscope. If the dispersed globules are appeared colorless with a blue background, the type of cream oil in water. Homogeneity the formulations were tested by visual appearance.

2.6.3. Viscosity Studies
The viscosity of the formulation was determined by Brookfield viscometer, using the spindle No. 7.

3. Result and discussion

3.1. Phenolic and Total Flavonoids content
It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. Flavonoids and phenolic compounds have been recently correlated with antioxidant properties of Stevia rebaudiana Bert. The mechanisms of action of flavonoids are through scavenging or chelating process [10]. Phenolic compounds are a class of antioxidant agents which act as free radical terminators [11]. The content of total phenols stevia extracts expressed as mg of gallic acid equivalents per gram of extracted stevia leaves, was noted respectively 24,726 — the number of flavonoids in analyzed stevia extract 296,945 mg Qu/g of extracted stevia leaves. The compounds such as flavonoids, which contain hydroxyls, are responsible for the radical scavenging effect in the plants [12].

3.2. DPPH Radical Scavenging Activity
The results of the antioxidant activity extract stevia leave test using DPPH were shown in Table 2. These results indicate that ascorbic acid has higher antioxidant activity with 10.12 µg / ml compared to extracts of 48.18 µg / ml. The extracts were found to be less active compared to the ascorbic acid. Antioxidants through their scavenging power are useful for the management of those diseases. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts [13]. DPPH assay is considered a valid accurate, easy and economical method to evaluate radical scavenging activity of antioxidants since the radical compound is stable and need not be generated [14].

| Sample                              | IC50 (µg/ml) |
|-------------------------------------|-------------|
| Vitamin C                           | 10.12       |
| Stevia rebaudiana Bert extract      | 48.18       |

3.3. Evaluation of Antioxidant Cream
The antioxidant Stevia leaves cream were evaluated for several physicochemical tests. The results were shown in table 3.

| Parameter     | Formulation               |
|---------------|---------------------------|
| Appearance    | yellowish and light brownish cream |
| Odor          | Aromatic                  |
| Homogeneity   | Homogenous                |
| pH            | 6                         |
| Spreadability | Good                      |
After feel Emollients
Removal Easily removed with water
Viscosity 370 cps

The cream showed brownish yellow colored with a pleasant odor. After application on the skin, it is not greasy and were easily washing with water and homogenous distribution by visual examination. The cream pH ranges from 5.50 - 6.62 which is good for skin. The emulsion type test results indicate the type O / W emulsion cream. Viscosity creams were 370 cps which indicated that the cream is easily spreadable by small amounts of shear. The cosmetic trend of using herbal ingredients for skin antioxidants is very popular because the application of this cream can protect the skin from UV damage.

4. Conclusion
This study determined that extract of Stevia leaves show antioxidant activity. Activity and the data for total phenolics and total flavonoids suggest that extract may be a good source of natural antioxidants which may be incorporated into a range of cosmetic and other products.

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