ABSTRACT

To evaluate the free radical scavenging activity of flavonoids containing extracts of Stevia leaf. Alcoholic. Successive butanolic and alcoholic extracts of leaves were examined for free radical scavenging activity using BHT (tert-butylhydroxytolune) as a positive control by in vitro models. Successive alcoholic extract showed remarkable free radical scavenging activity. The IC\textsubscript{50} was found to be 140 µg and 76 µg of successive alcoholic and BHT respectively in DPPH (2,2-diphenyl-1-picrylhydrazyl) assay method. The Dot-Blot test on silica layers also showed the scavenging activity of free radical of flavonoids containing extracts. The successive alcoholic extract shown significant antioxidant activity.

KEYWORDS : Antioxidant, flavonoids, Stevia and DPPH.

INTRODUCTION:

\textit{Stevia rebaudiana} (Bert) is a shrub belonging to the Compositae family, which his indigenous to the northern region of South Americal. It is an annual herb, 1-1 ½ fee in height, stem puberulous leaves opposite, ob lanceolate, crenulate flower heads very small, whitish in corymb. Leaves have a sugary flavour\textsuperscript{1}. From the literature survey it was found that the stevia leaf contains flavonoids. The flavonoids are natural products, which have been shown to possess various biological properties related to antioxidant mechanism\textsuperscript{2,3,4} hence an attempt was made to investigate antioxidant activity for flavonoids containing extracts.

MATERIALS AND METHODS

Preparation of the extracts:

The leaves of \textit{Stevia rebaudiana} were collected from the Belvatagi farm, Dharwad district and the same was authenticated by Dr. B. D. Huddar, Professor & Head, Dept. of Botany, H.S. Kotambri Science Institute, Hubli. The dried powdered leaves were exhaustively extracted with 95% ethanol in a soxlet apparatus and also extracted successively with pet ether, butanol, ethyl acetate and alcohol. The extracts were further concentrated in vacuum under pressure using rotary flash evaporator and dried in desicator. Qualitative chemical tests were performed for all extracts for identification of various phytoconstituents as shown in table 1. Alcoholic, successive alcoholic and butanolic extracts showed the presence of flavonoids and the same were used for screening free radical scavenging activity at a concentration of 2 mg/ml. in methanol.
**Free radical scavenging activity**: Scavenging of 1, 1-diphenyl 1-2-picrylhydrazyl radicals

The free radical scavenging capacity of alcoholic, successive alcoholic and butanolic extracts of stevia leaves was tested by its ability to bleach the stable DPPH. A stock solution of DPPH was prepared to give a concentration of 90µM. This stock solution was used to measure the antiradical activity. Decrease in the absorbance in the presence of extracts at different concentrations was noted after half an hour at 515 nm using Jasco UV 530 Spectrophotometer. The difference in absorbences between the test and the control was calculated and expressed as percent scavenging of DPPH radical. BHT solution of 2mg/ml in methanol was used as a reference standard.

Assessment of free radical scavenging activity was done by calculating % RSC as shown in table no.02. The IC50, values which represented the concentrations of extract that caused 50% neutralization, were determined by linear regression analysis and shown in figure no.1

**Dot – Blot test on TLC Silica layer method:**

For fast screening of flavonoids containing extracts of RSC (radical scavenging capacity), the Dot – Blot test of TLC silica layers, stained with free radical DPPH was used. Alcoholic, successive alcoholic and butanolic extracts were used for scavenging free radicals of 90µM DPPH solution. Appropriate amount of flavonoids containing extracts (5-10µl) were placed on silica gel plates and chromatographed in the solvent system ethyl acetate: formic acid : glacial acetic Acid: water in the ratio of 05:0. 25:0. 25:1.35 (water layer was removed after proper mixing of the solvents). After drying the mobile phase, the staining on silica layers was carried out by spraying the layer with 90µM solution of DPPH using sprayer.

This method is based on monitoring the transformation of purple colored DPPH into its reduced yellow colored form diphenylpicrylhydrazine (DPPH-H)

**RESULTS AND DISCUSSION:**

Qualitative chemical analysis of various extracts showed the presence of carbohydrates, flavonoids, steroids, triterpenoids, tannins, proteins, glycosides and amino acids. Successive alcoholic extract of *Stevia rebaudiana* acts as a potential free radical scavenger as indicated by DPPH is a relatively stable free radical. The assay is based on the measurements of the scavenging ability of antioxidants towards the stable radical DPPH. DPPH radicals react with suitable reducing agents, the electrons become paired off and the solution loses colour stoichometrically depending on the number of electrons taken up. The IC50 value of it was found at 140µg compared with the IC50 76µg value BHT.

The Dot-Blot test on TLC silica layers showed antioxidant activity on extracts containing flavonoidal components. The dot blot test on silica layers also showed the scavenging activity for DPPH on these extracts. Successive alcoholic extract has shown better activity as compared to other extracts. The results obtained showed that the successive extract of alcohol has a better antioxidant activity than other extracts. Further investigations are needed by using other models and further isolation of...
bioactive flavonoids responsible for antioxidant activity.

**Table No.1**  
Qualitative chemical analysis of various extracts

| Phyto-Constituents | Alcoholic Extract | Successive Extractions | PE | B.Nol | E.A | Alcohol | Water |
|--------------------|-------------------|------------------------|----|-------|-----|---------|-------|
| Carbohydrates      | +                 | -                      | -  | -     | +   | +       |       |
| Flavonoids         | +                 | -                      | +  | -     |     | +       | -     |
| Alkaloids          | -                 | -                      | -  | -     | -   | -       |       |
| Steroids           | +                 | +                      | -  | -     | -   | +       | -     |
| Triterpenoids       | +                 | +                      | -  | -     | -   | -       |       |
| Tannins            | +                 | -                      | -  | -     | -   | -       |       |
| Proteins & amino acid | -               | -                      | +  | -     | -   | -       | +     |
| Glycosides         | +                 | -                      | +  | +     | +   | +       | -     |

**Keywords:**  
PE = Petroleum Ether Extract (40-60°C)  
B.Nol = Butanol Extract  
EA = Ethyl Acetate Extract

**Table No.2**  
Percentage of neutralization of DPPH using extracts and BHT in the DPPH assay

| Sr. No. | µl | Alcoholic | S.Butanolic | S.Alcoholic | BHT |
|---------|----|-----------|-------------|-------------|-----|
| 1       | 10 | 22.62     | 18.69       | 26.88       | 44.26 |
| 2       | 25 | 22.70     | 23.86       | 32.3        | 51.85 |
| 3       | 50 | 29.26     | 26.02       | 44.34       | 55.92 |
| 4       | 75 | 34.37     | 29.13       | 53.55       | 59.80 |
| 5       | 100| 43.16     | 29.76       | 63.31       | 60.95 |
| 6       | 125| 48.03     | 36.45       | 69.90       | 65.67 |
| 7       | 150| 52.52     | 39.96       | 76.03       | 69.37 |
| 8       | 175| 59.10     | 46.32       | 78.05       | 70.45 |
| 9       | 200| 62.62     | 51.01       | 79.52       | 70.88 |
**Figure No.2:** Histogram showing % RSC of Alcohol, s.butanolic, s.alcoholic

**Figure No.1:** Graph showing % RSC of alcoholic, successive butanolic, alcoholic and BHT as a positive control.