



## MOLECULAR AND MICROBIOLOGICAL ASSESSMENT OF STEVIA (*Stevia rebaudiana* BERTONI) LEAF EXTRACTS FOR ANTI-BACTERIAL AND ANTI-MUTAGENIC ACTIVITIES

Mohamed M.A. Elashtokhy\* and Heba S.A. Taha

Genet. Dept., Fac. Agric., Zagazig Univ., Egypt

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**ABSTRACT:** The purpose of the study is to assess the anti-bacterial and anti-mutagenic activities of *Stevia rebaudiana* leaf extracts on some pathogenic bacteria. Two extracts had been tested which prepared by two different solvents. Acetone extract was more effective as anti-bacterial than ethyl alcohol extract. Acetone extract showed high activity against Gram-positive bacteria which recorded (20 mm) while it recorded (18 mm) against Gram-negative bacteria. The study enhanced the antibacterial activity of *Stevia rebaudiana* leaf extracts using different solvents. The effectiveness of stevia extract as anti-mutagenic agent on *Pseudomonas aeruginosa* was also evaluated. The results of induction of prophage F<sub>116</sub> from *P. aeruginosa* after treated with different concentrations from each stevia extracts with ascorbic acid as negative control showed that stevia extract has no mutagenic activity on the tested bacteria. Moreover, the concentrations of ethyl methanesulfonate (EMS) when used as positive control showed a mutagenic effect. However, 100 mg/ml of stevia extract had reduced mutagenic activity of EMS. This finding suggested that stevia leaf extract may be playing a remarkable role in anti-mutagenic response. Susceptibility of the bacterial strain to exposure to different mutagenic and non-mutagenic agents has been assessed by analysis the variation of protein profile banding pattern (SDS-PAGE). The comparison between banding pattern when bacteria treated with each of stevia leaf extract, ascorbic acid and EMS, demonstrated that concentration 100 mg/ml of stevia leaf extract had significant effect to reduce the effect of the EMS as mutagenic agent in bacteria. Results from this study suggest that natural products like stevia leaf extract could be as an effective agent reducing the effect of the mutagenic agents. In addition to the antimicrobial properties which can be applied in the medical field that requires further studies and development.

**Key words:** Anti-bacterial, anti-mutagenic, *Stevia rebaudiana*, SDS-PAGE.

## INTRODUCTION

*Stevia rebaudiana* (Bertoni) is a herbal plant permanently of genus *Stevia* Cav., which consists of 230 species of herbaceous approximately, diterpene glycosides produced from leaf of stevia (stevioside and rebaudiosides). In addition to properties of sweetener, it has own medicinal values and uses. Rebaudioside is a more interesting from the glycosides produced in its leaf because of flavour profile is the most desirable, while stevioside is responsible for aftertaste bitterness (Yadav *et al.*, 2011). *Stevia*

*rebaudiana* (Bertoni) is from Asteraceae family as herbaceous perennial plant and Paraguay is native source (South America). The sweetener present in leaf and stem tissues of plant called stevioside, which was first used as a sugar substitute in the early 1970s formed for the commercializing purpose (Kinghorn and Soejarto, 1985).

Indians used stevia also as a sweetener and as a medicinal plant (Anju *et al.*, 2014). *Stevia* considered as particularly a substance strengthening the heart, regulating blood pressure and the circulatory system. Also sweet stevia leaf is

\*Corresponding author: Tel.: +20117101616

E-mail address: mohamed\_mam82@yahoo.com

used in liver disease, gastric hyperacidity, stomachache and as a mild agent stimulating the nervous system. It shows anti-bacterial and anti-fungal properties (Katarzyna and Zbigniew, 2016).

Stevia is counted as a natural source of sweetener without caloric, also as alternate now available to the diet consumers for artificial sweeteners. Randi (1980) studied the use of Stevia, as a sweetener material without caloric, which produces stevioside, which does not metabolize in the human body. Stevioside and stevia extracts contains glycosides produced by the leaf which have high content of sweeter than sucrose. For that reason, it can be utilized and to replace than with sucrose (Sekaran *et al.*, 2007).

Different parts of *S. rebaudiana* plant were known to have antioxidant activity and anti-bacterial activity (Singh *et al.*, 2012; Bender *et al.*, 2015). *S. rebaudiana* (Bertoni) has been used as agent for anti-hyperglycemic for the curing of diabetes for decades. Stevia with insulin had a role in controlling the level of glucose (Bhasker *et al.*, 2015).

*Stevia rebaudiana* leaf extracts demonstrated anti-bacterial, anti-fungal, anti-yeast and anti-tumor activity (Sathishkumar *et al.*, 2008). Microbiological assessments of stevia plant extract against positive and negative pathogenic species using different solvents has a few reports (Takai, 1985; Tomita, 1997; Tadhani *et al.*, 2006). Few reports concerning the anti-microbial and anti-tumor activity of *Stevia rebaudiana* were conducted (Tadhani and Subash 2006). Presently many research centers have being investigation in nutritive and health-promoting properties of stevia (Katarzyna and Zbigniew, 2016).

*Stevia pilosa* and *Stevia eupatoria* are plants used for various purposes in traditional medicine. The methanolic extracts obtained from leaf studied for the anti-mutagenic effect using the Ames test with and without metabolic activation against three mutagens. The results found that the two species of stevia have anti-mutagenic potential by reducing the mutations induced by the tested mutagenesis (Carino *et al.*, 2007).

Some plant extracts also showed anti-mutagenic potential. Here as *Bauhinia galpinii* N.E.Br. (Fabaceae) and especially *Chlerodendrum myricoides* (Hochst.) appear to have anti-mutagenic properties which considered of a particular interest as it may be assumed that these anti-mutagenic natural substances are able to lower the cancer risk from everyday exposures to environmental mutagenesis (Verschaeve and Vanstaden, 2008).

Anti-mutagenic properties flavonoids extracts of three plants *Gratiola officinalis* L., *Helichrysum arenarium* L. and *Zea mays* L. were investigated. Performed has been tested by counting the micronucleus in white mice blood. The selected extracts reduce the number of micronucleus (Durnova and Kurchatova 2015).

The broad spectrum anti-oxidant and anti-mutagenic activities have been evaluated. Reactive oxygen species ROS may function a main role as endogenous initiators of degenerative processes, like DNA damage and mutation. Therefore, the discovery and the exploration of compounds processing anti-oxidant and anti-mutagenic properties are of great practical and have a therapeutic significance (Zahin *et al.*, 2010).

The present study was undertaken for further analysis using *Stevia rebaudiana* to evaluate the anti-mutagenic and anti-bacterial activities which could be interesting.

## MATERIALS AND METHODS

### Stevia Plant (*Stevia rebaudiana* Bertoni)

Stevia leaves have been obtained from Sugar Crop Research Institute (SCRI), Agriculture Research Center, Giza, Egypt. The leaves were washed with sterile water, dried at room temperature and then blinded to give a homogenate material and stored in air tight bottles.

### Bacterial Assay

All the experiments had been done at Microbial Genetics Lab, Genetics Department, Faculty of Agriculture, Zagazig University, Egypt.

### Test organisms

Gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis* and *Listeria monocytogenes*) and Gram negative bacteria (*Escherichia coli*, *Serratia marcescens* and *Salmonella typhi*), were kindly obtained from Laboratory of Microbiology, Department of Agricultural Microbiology, Faculty of Agriculture, Zagazig University, Egypt.

Bacteriophage F<sub>116</sub> and the *Pseudomonas aeruginosa* bacterial strains were obtained kindly from Prof. M. Day, University of Wales, Cardiff, United Kingdom. Strains were routinely grown on Nutrient agar (NA) and nutrient broth (NB). Phage F<sub>116</sub> that has been used in this study is a generalized transducing phage (Holloway *et al.*, 1960). *Pseudomonas aeruginosa* L. lysogenic bacterial had been used to assess the mutagenic activity of the plant.

The cultures were stored at 4°C on NA plates as single colonies. All strains were sub-cultured on fresh media monthly with standard technique of phage titration.

### Preparation of Plant Extract

100 g of dried leaf powder of *Stevia rebaudiana* was flooded in 100 ml of organic solvent acetone and ethyl alcohol with concentration 60% separately in a flask. It was incubated at room temperature for 2.3 days at 150rpm in an orbital shaker, filtered and re-soaking again. Finally the extract was filtered using filter membrane (0.2 µm, whatman). Keeping to dryness at 40°C in hot air oven. The extract was dissolved in 0.25% Dimethyl Sulphoxide (DMSO) to a concentration of 100 mg/ml. This solution has been sterilized by filtration and then a stock of stevia extract was prepared. Concentrations of stevia which have been prepared were 10, 25, 50, 75, 100, 125, 150 and 200 mg/ml.

### Assay for antibacterial activity

#### Preparation of inoculum

Fresh cultures for experiments were prepared by transferring a loopful of culture to 10ml of nutrient broth and incubated overnight for bacterial proliferation at 37°C for *Staphylococcus aureus*, and at 30°C for *E. coli* and 25°C for other strains. NA and NB were prepared

according to manufacturer's instructions (Sigma). Stock cultures were maintained at 4°C on slants nutrient agar.

#### Disc diffusion method

Disc diffusion method was performed according to (Bauer *et al.*, 1966) to screen the anti-bacterial activity with some modifications. The sensitivity test of the acetone and ethyl alcohol extracts were determined using agar disc diffusion method. Different concentrations (10, 25, 50, 75, 100, 125, 150 and 200 mg/ml) of stevia extracts were prepared, the antibiotic positive control tetracycline (30 mg/ml), and sterile distilled water as a negative control. Briefly, petri dishes containing 20ml of culture medium (3 replicates) under aseptic conditions. After performing the solidification of the medium and incubation for two days in order to ensure sterility, aliquots (0.1ml of broth culture ~ 10<sup>8</sup> CFU/ml<sup>-1</sup>) of bacterial suspensions was spreaded over the surface of the above mentioned medium by soft agar as a layer; after the agar had solidified according to (Somayeh *et al.*, 2014) 20 µl of each dilution was impregnated into sterile, blank discs 6mm in diameter and placed at equidistance on the agar surface. 5 µl of extract was spotted alternately on both sides of the discs and allowed to dry before the next 5 µl was spotted to ensure precise impregnation. The investigated stevia extracts at various concentrations were added to each disc. The plates were incubated for a time period at 37°C for *Staphylococcus aureus*, 30°C for *E. coli* and 25°C for other strains. The antibacterial activity of the plant extract was determined by measuring the diameter of the inhibition zone. The antibacterial assay for each extracts was performed out with 3 replicates.

### Mutagenic Activity of Stevia Extract

#### Prophage induction assay

To separated cultures of *Pseudomonas aeruginosa* lysogen strain has been treated with the concentrations 10, 25, 50, 75, 100, 125, 150 and 200 mg/ml from stevia leaf extract, then incubated at 30°C overnight on shaker and centrifugated at 4000rpm for 30min. Number of Cfu/ml and Pfu/ml were calculated. Fold increase (FI) was calculated by dividing Pfu/ml of each concentration on Pfu/ml of control

Mutagenic index (MI) was detected according to Heinemann (1971) any compound give 3 fold increases in prophage induction than control should be mutagenic agent.

#### Negative control

One gram of ascorbic acid (vitamin C) from Sigma dissolved in 100ml of distilled water as a stock. Make sterilization by filtration. Concentrations 10, 25, 50, 75, 100, 125, 150 and 200 mg/ml from ascorbic acid were added to NB inoculated with *P. aeruginosa*. Incubated at 30°C overnight on shaker and centrifugated at 4000 rpm for 30min. Number of Pfu/ml was calculated. (FI) was calculated and mutagenic index (MI) was detected.

#### Positive control

Ethyl methanesulphonate (EMS) has been used as a positive control. The same concentrations preceding that described in the negative control test were used. (FI) was calculated and mutagenic index (MI) was detected.

#### Anti-mutagenic Activity of Stevia Extract

100 mg/ml of stevia leaf extract was added to liquid culture from *P. aeruginosa* contain concentrations of 10, 25, 50, 75, 100, 125, 150 and 200 mg/ml from EMS separately, incubated these mixture at 30°C for overnight on shaker and centrifugated at 4000rpm for 30 min. Number of Pfu/ml was calculated. (FI) was calculated and mutagenic index (MI) was detected.

#### Protein profile SDS-PAGE

Each concentration from stevia leaf extract, ascorbic acid (Vitamin C) as a negative control, (EMS) as a positive control and mixture from 100 mg/ml stevia leaf extract with EMS concentrations have been added to liquid culture from *P. aeruginosa* bacterial strain. After incubation at 25°C for overnight on shaker, 1 ml from incubated liquid culture was centrifuged to collect the pellet to prepare the protein sample. Gel electrophoresis (SDS-PAGE) was performed following the method of Laemmli (1970). The samples were prepared in precautionary conditions with  $\beta$ -mercaptoethanol using 12% separating gel and 5% stacking gel, and

molecular weight markers were used to determine molecular mass. Electrophoresis was performed at 200 V for 5 hr. The gel was stained with coomassie brilliant blue R-250.

## RESULTS AND DISCUSSION

#### Anti-bacterial Activity

The extracts of *Stevia rebaudiana* showed a significant effect as antibacterial activity as shown in Table 1. Two extracts, *i.e.* acetone and ethyl alcohol extract were tested with all of gram-positive and gram-negative bacteria. Acetone extract showed a higher anti-bacterial activity, followed by ethyl alcohol extract. The largest inhibition zones were observed with 200 mg/ml for acetone extract against gram-positive bacteria *Listeria monocytogenes* (20 mm), *Staphylococcus aureus* and *Bacillus subtilis* (18 mm). While with ethyl alcohol extract was (18 and 17 mm) respectively. Also with gram-negative bacteria, acetone extract was very effective against *Salmonella typhi* (18 mm), *Serratia marcescens* (15 mm) and *Escherichia coli* (16 mm) compared with ethyl alcohol extract (16 mm) with *Salmonella typhi* and recorded (14 mm) with other gram-negative bacteria. The minimum inhibition zone was observed with 10 and 25 mg/ml in *Stevia rebaudiana* leaf extract against all gram negative bacteria (7 mm) with ethyl alcohol extract while recording for *Escherichia coli* and *Salmonella typhi* was (8 mm) with acetone extract. In the present study, it has been also observed that gram positive bacteria were more sensitive than gram negative bacteria in the selected plant extracts. The higher anti-bacterial activity of the acetone extract compared with ethyl alcohol extract may be due to the greater solubility of the extract in these organic solvents (De Boer *et al.*, 2005). Manish *et al.* (2006) reported that hexane extract of *Sativa rebaudiana* leaf showed higher activity compared to methanol ethyl acetate extract against microorganism tested. Sathishkumar *et al.* (2008) also recorded high anti-bacterial activity for acetone extracts of *Stevia rebaudiana* leaf. Moreover, investigated the anti-bacterial activity obtained from *Stevia rebaudiana* extraction was higher in ethanol when compared between ethanol and methanol solvents (Sunitha *et al.* 2015).

**Table 1. Antibacterial activities of the *Stevia* leaf extracts**

Concentrations	Zone of inhibition (mm)															
	10 mg/ml		25 mg/ml		50 mg/ml		75 mg/ml		100 mg/ml		125 mg/ml		150 mg/ml		200 mg/ml	
Solvents	Acetone	Ethyl alcohol	Acetone	Ethyl alcohol	Acetone	Ethyl alcohol	Acetone	Ethyl alcohol	Acetone	Ethyl alcohol	Acetone	Ethyl alcohol	Acetone	Ethyl alcohol	Acetone	Ethyl alcohol
<b>Test organisms</b>	<b>Gram positive bacteria</b>															
<i>Staphylococcus aureus</i>	9	8	11	8	12	10	15	12	17	15	17	16	18	16	18	17
<i>Bacillus subtilis</i>	10	9	12	10	14	10	16	11	17	12	17	14	18	16	18	17
<i>Listeria monocytogenes</i>	8	7	10	8	12	10	14	12	16	14	18	15	18	17	20	18
	<b>Gram negative bacteria</b>															
<i>Escherichia coli</i>	8	7	8	7	10	7	10	8	11	11	13	12	14	13	16	14
<i>Serratia marcescens</i>	7	7	9	7	9	8	10	10	12	11	13	12	13	13	15	14
<i>Salmonella typhi</i>	8	7	8	7	10	9	12	11	14	12	15	13	17	15	18	16

### Prophage Induction Assay

The induction of prophage F<sub>116</sub> is shown in Table 2. The obtained results show a low increase in prophage F<sub>116</sub> induction from *P. aeruginosa* lysogen strain. The titer of phage ranged from  $1.47 \times 10^5$  up to  $2.29 \times 10^5$  Pfu/ml as a result of using the concentrations from 10mg/ml up to 200mg/ml. However, according to Heinemann (1971) the mutagenic index (MI) in all used concentrations was less than 3. So the concentrations of stevia that used have no mutagenic activity.

The same results have been obtained upon using ascorbic acid as a negative control (Table 2). The MI values were less than 3 by using concentrations from ascorbic acid similar to those used.

The EMS as a mutagenic agent was used in this investigation as a positive control. All the used concentrations showed a mutagenic effect (Table 2). Whereas, the mutagenic index ranged from 3.00 at 10 mg/ml up to 11.98 at 200 mg/ml.

### Anti-mutagenic Activity

Results in Table 3 show the anti-mutagenic response of stevia. 100 mg/ml of stevia leaf extract reducing the mutagenic activity when has been used with concentrations from EMS.

Mutagenic index (MI) dropped from 11.98 at 200 mg/ml of EMS up to 3.96. These results appear that this plant has anti-mutagenic activity in the range of concentrations that are used in this study. This anti-mutagenic effect has been attributed to the anti-oxidant properties of that plant as determined by both (Carino *et al.*, 2007; Kaushik *et al.*, 2010; Javarini and Angelini, 2013; Ruiz-Ruiz *et al.*, 2015).

Anti-mutagenic studies are a good alternative way for obtaining important information to explore the possibilities for reducing the genotoxic risk exposure to genotoxic compounds. Many studies of natural plants, such as *Stevia pilosa*, *Stevia eupatoria* and *Castela texana*, have exhibited antimutagenic and antigenotoxic properties (Reyes-Lopez *et al.*, 2005 ; Carino *et al.*, 2007).

### Protein Profile SDS-PAGE

Analysis of the protein banding pattern SDS-PAGE packages produced by the treatment of different concentrations of stevia extract leaf, ascorbic acid (vitamin C), ethyl methylesalphonate (EMS) and 100mg/ml of stevia extract with each of concentrations from EMS represented in Fig. 1 A, B, C and D. A wide variation in density and number of banding pattern compared to control was shown. (Fig. 1A) showed 18 protein

**Table 2. Prophage F<sub>116</sub> induction from the *P. aeruginosa* lysogen strain by stevia extract, ascorbic acid and EMS**

Co.	Stevia extract			Ascorbic acid (Negative control)			EMS (positive control)		
	Pfu/ml (10 <sup>5</sup> )	Mutagenic index (MI)	Fold increase (FI)	Pfu/ml (10 <sup>5</sup> )	Mutagenic index (MI)	Fold increase (FI)	Pfu/ml (10 <sup>5</sup> )	Mutagenic index (MI)	Fold increase (FI)
Control	1.47 ± 0.01	0.00	-	1.52 ± 0.01	0.00	-	1.34 ± 0.03	0.00	-
10 mg/ml	1.63 ± 0.02	1.10	-	1.66 ± 0.02	1.09	-	4.10 ± 0.15	3.00	+
25 mg/ml	1.75 ± 0.04	1.19	-	1.74 ± 0.05	1.14	-	4.42 ± 0.17	3.29	+
50 mg/ml	1.82 ± 0.02	1.23	-	1.86 ± 0.07	1.22	-	5.62 ± 0.19	4.19	+
75 mg/ml	1.87 ± 0.03	1.27	-	2.00 ± 0.04	1.15	-	7.32 ± 0.19	5.46	+
100 mg/ml	1.94 ± 0.05	1.31	-	2.13 ± 0.08	1.31	-	9.09 ± 0.25	6.78	+
125 mg/ml	2.01 ± 0.04	1.36	-	2.20 ± 0.03	1.44	-	12.14 ± 0.25	9.05	+
150 mg/ml	2.18 ± 0.05	1.48	-	2.24 ± 0.04	1.47	-	13.22 ± 0.30	9.86	+
200 mg/ml	2.29 ± 0.06	1.55	-	2.38 ± 0.06	1.56	-	16.06 ± 0.35	11.98	++

Value is mean of three replicates ± SD.

- = Fold increase &lt; 3

+ = Fold increase &gt; 3

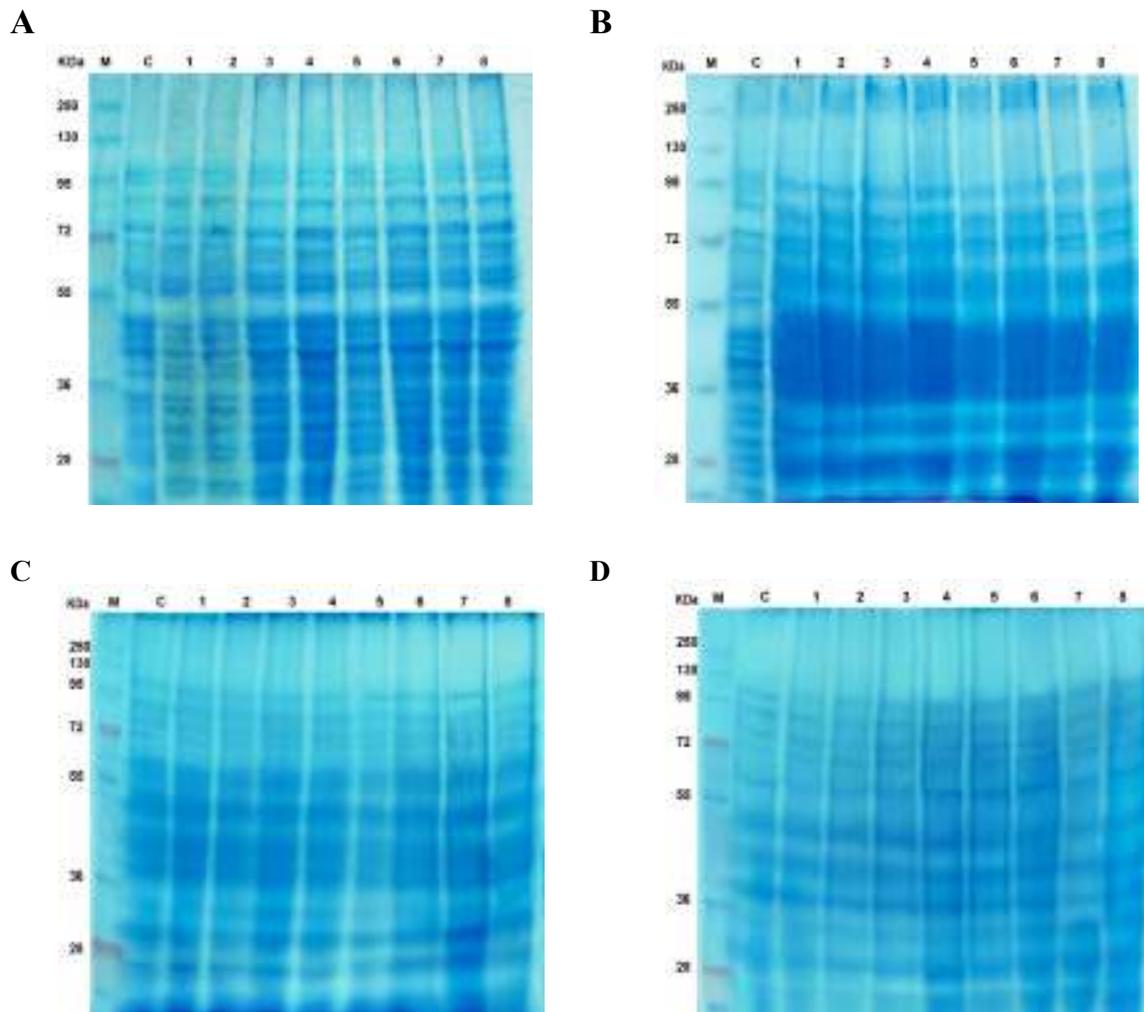
**Table 3. Anti-mutagenic activity of 100mg/ml of stevia leaf extract with concentrations of EMS**

Co. of EMS	Pfu/ml (10 <sup>5</sup> )	Mutagenic index (MI)	Fold increase (FI)
Control	1.44 ± 0.10	0.00	-
10 mg/ml	2.12 ± 0.20	1.47	-
25 mg/ml	2.54 ± 0.10	1.76	-
50 mg/ml	2.67 ± 0.30	1.85	-
75 mg/ml	3.11 ± 0.20	2.15	-
100 mg/ml	3.56 ± 0.30	2.47	-
125 mg/ml	4.42 ± 0.10	3.06	+
150 mg/ml	5.12 ± 0.20	3.55	+
200 mg/ml	5.71 ± 0.10	3.96	+

Value is mean of three replicates ± SD.

- = Fold increase &lt; 3

+ = Fold increase &gt; 3



**Fig. 1. SDS-polyacrylamide gel electrophoresis of protein in *P. aeruginosa***

- (A) Lane M: PageRuler™ protein ladder, Lanes C: Control Lanes (1:8) represent different concentration of stevia extract leaf (10, 25, 50, 75, 100, 125, 150 and 200mg/ml) respectively, treated with *P. aeruginosa*.
- (B) Lane M: PageRuler™ protein ladder, Lanes C: Control Lanes (1:8) represent different concentration of EMS (10, 25, 50, 75, 100, 125, 150 and 200mg/ml) respectively, treated with *P. aeruginosa*.
- (C) Lane M: PageRuler™ protein ladder, Lanes C: Control Lanes (1:8) represent different concentration of Ascorbic acid (10, 25, 50, 75, 100, 125, 150 and 200mg/ml) respectively, treated with *P. aeruginosa*.
- (D) Lane M: PageRuler™ protein ladder, Lanes C: Control Lanes (1:8) represent different concentration of EMS (10, 25, 50, 75, 100, 125, 150 and 200mg/ml) respectively, with 100mg/ml stevia extract treated with *P. aeruginosa*.

bands from ( $\approx 100$ : 10KDa) in all treated with stevia concentration and control, that was compatible with (Nadakumar *et al.*, 2002) When compared between eleven *Pseudomonas* isolates for their total cell proteins separated through sodium dodecyl sulphate polyacrylamide gel electrophoresis. However, major differences were detected in the second treatment among treated with EMS, (Fig. 1B) revealed a unique regions with high density on banding pattern especially in the area of ( $\approx 55$ : 30KDa) which appeared more densely which may be a reflection of the effect of mutagenic agent.

Despite clear variation observed in banding pattern, similarity effects was also observed in each concentrations of EMS beginning of the least concentration compared to control. On the other side, ascorbic acid (Vitamin C) has been used as a negative control, (Fig. 1C) showed a dense patterns compared with control, but similar with them in molecular weight and numeration. Finally, the effect of the 100 mg/ml of stevia extract with each concentrations of EMS was very interesting (Fig. 1D), because the banding pattern has appeared naturally again compared to control, suggesting that stevia leaf extract reduced the mutagenicity effect of the EMS. Therefore stevia leaf extract has a significant role in reducing the effect of the mutagenic activity. This is based on the relationship between anti-microbial, anti-oxidant, and anti-mutagenic activities which has been studied from (Burcu *et al.*, 2014). Also according to (Katarzyna *et al.*, 2015) stevia extracts as a multifunctional source of natural antioxidants. These results support the idea that stevia plant may have an anti-mutagenic activity. Stevia leaf extract could be considered as an effective agent (Theophilus *et al.*, 2015). This may be due to antioxidants properties of this plant.

## Conclusion

This study points to the probable antimicrobial and anti-mutagenic activity of some solvent extracts of *Stevia rebaudiana* leaf. There is a need for further investigation of this plant in order to identify and isolate its active gradients. The results of the study may be remarkable to be used in the medical field and will also need to be confirmed using in vivo models. The anti-mutagenic activity may be due to the strong antioxidant properties of stevia which make it very interesting to using stevia extracts on a large scale to improve industrial food properties.

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## التقييم الجزيئي والميكروبيولوجي لمستخلصات أوراق الأستيفيا (*Stevia rebaudiana* Bertoni) للأنشطة المضادة للبكتيريا والمضادة للطفور

محمد ممدوح عبدالنواب الأشطوخي - هبه سيد أحمد طه

قسم الوراثة - كلية الزراعة - جامعه الزقازيق - مصر

الغرض من هذه الدراسة هو تقييم الأنشطة المضادة للبكتيريا والمضادة للطفور من مستخلصات أوراق الأستيفيا على بعض البكتيريا المسببة للأمراض، تم اختبار اثنين من المستخلصات التي أعدت بأثنين من المذيبات المختلفة، كان مستخلص الأستيون أكثر فعالية كمضاد للبكتيريا من مستخلص الكحول الإيثيلي، أظهر مستخلص الأستيون نشاطاً أعلى ضد البكتيريا الموجبة لجرام والتي سجلت (٢٠ملم) بينما سجلت البكتيريا السالبة لجرام (١٨ملم)، وعززت الدراسة الأنشطة المضادة للبكتيريا من مستخلصات أوراق الأستيفيا باستخدام المذيبات المختلفة، كما تم تقييم فعالية مستخلص الأستيفيا كعامل مضاد للطفور على بكتيريا *P. aeruginosa*، أظهرت نتائج إنتاج فاجات الـ F<sub>116</sub> من بكتيريا *P. aeruginosa* بعد المعاملة بتركيزات مختلفة من مستخلص الأستيفيا وحمض الأسكوربيك أن مستخلص الأستيفيا ليس له نشاط طفرى على البكتيريا المختبرة، وعلى الجانب الآخر فإن تركيزات الأيثيل ميثيل سلفونيت أظهرت التأثير الطفرى، ومع ذلك فإن ١٠٠مجم/مل من مستخلص الأستيفيا خفضت النشاط الطفرى للأيثيل ميثيل سلفونيت، وتشير هذه النتيجة إلى أن مستخلص أوراق الأستيفيا قد تلعب دوراً ملحوظاً في الاستجابة المضادة للطفور، قابلية السلالة البكتيرية للتعرض لمختلف العوامل المطفرة وغير المطفرة قد تم تقييمها من خلال تحليل الاختلافات في نمط حزم البروتين الناتجة من التفريد الكهربى، أظهرت المقارنة بين نمط الحزم عند معاملة بكتيريا *P. aeruginosa* مع كل من مستخلص أوراق الأستيفيا، حمض الاسكوربيك والإيثيل ميثيل سلفونيت، أن تركيز ١٠٠مجم/مل من مستخلص أوراق الأستيفيا كان له تأثير كبير لتقليل تأثير الإيثيل ميثيل سلفونيت كعامل مطفر فى البكتيريا، وتشير نتائج هذه الدراسة إلى أن المنتجات الطبيعية مثل مستخلص أوراق الأستيفيا يمكن أن تكون عامل فعال للحد من تأثير العوامل المطفرة، بالإضافة إلى الخصائص المضادة للبكتيريا والتي يمكن تطبيقها في المجال الطبي الذي يتطلب المزيد من الدراسات والتطوير.

المحكمون:

١- أ.د. محمد سراج الدين عبدالصبور  
٢- أ.د. سعيد سعد سليمان

أستاذ الوراثة المتفرغ - كلية الزراعة - جامعة بنها.  
أستاذ الوراثة المتفرغ - كلية الزراعة - جامعة الزقازيق.