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Original Article

ANTIOXIDANT ACTIVITY, TOTAL PHENOLIC AND FLAVONOID CONTENTS, AND CYTOTOXICITY EVALUATION OF *BOUGAINVILLEA XBUTTIANA*

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ABSTRACT

Objective: *Bougainvillea xbuttiana* is widely distributed in Mexico and it used as an analgesic in folk medicine. The aim of this study was to investigate the effect of five ethanolic extracts of *Bougainvillea xbuttiana* from different colours on cell and to quantify the potential of antioxidant activity, the total phenolic and flavonoids contents, all these in order to find possible sources for future novel antioxidant and pharmaceutical formulations.

Methods: The effect of aerial part (flowers) of *B. xbuttiana* extracts from different colours such as orange-1 (*BxbO1*), orange-2 (*BxbO2*), rose (*BxbR*), violet (*BxbV*) and white (*BxbW*) were determined on cells by viability and vacuolization assays. The cellular viability activity was determined by taking the lyses percentage and the vacuoles formation. The antioxidant activity was performed by 2,2´-diphenyl-1-pierylhydrazyl (DPPH) and ferric reducing power (FRAP) assays. The total yield of phenolic and flavonoids contents was determined using spectrophotometer methods.

Results: In this study, the effect of *Bxb* extracts on cells was shown by cytotoxicity percentage and vacuoles formation. The highest cytotoxic activity was observed when HeLa cells were exposed to *BxbO1* and *BxbO2* extracts. With respect to vacuoles formation the highest percentage of vacuoles formation was observed when the HeLa cells were exposed to *BxbR*. The phytochemical screening of ethanolic extracts of *B.xbuttiana* from different colour revealed the antioxidant activity with ranking order for the antioxidant activity index was *BxbO2* = *BxbR* > *BxbW* > *BxbO1* > *BxbV*. The ranking order for the reduction capacity was *BxbO2* = *BxbR* > *BxbW* = *BxbV* > *BxbO1*. The total phenolic content ranged from 14.68 to 28.89 of dry weight of extract, expressed as gallic acid equivalents, and the ranking order was *BxbO2* > *BxbR* > *BxbW* > *BxbV* > *BxbO1*. The total flavonoid contents varied from 59.13 to 129.38 expressed as EQmg/g dry weight and the ranking order was *BxbV* > *BxbO2* = *BxbO1* > *BxbR* > *BxbW*.

Conclusions: Data from present results show that aerial part (flowers) of *Bougainvillea xbuttiana* possesses significant free radical scavenging properties colour-dependent manner, and clear correlation exists between the strong antioxidant activity, and phenolic, and flavonoids contents. The results suggest that *Bxb* can be regarded as promising candidates for natural plant sources of antioxidants with high value.

Keywords: *Bougainvillea xbuttiana*, Antioxidant, Flavonoids, Phenols, Cytotoxic.

INTRODUCTION

Since the beginnings of civilization, humans have used natural products for to cure the series of diseases. Distinct investigations described that various pharmaceutical agents have been part of traditional healthcare for thousands of years and recently have gained a lot of importance in the field of pharmacological industries. The increasing interest in evaluating the antioxidant properties to the drug discovery process has been frequently focused on the screening of plant extracts as source of novel drug candidates that contribute to the development of many diseases [1 - 2]. In general natural oxidants or phytochemical antioxidants are secondary metabolites of plants. These metabolites have important biological and pharmacological activities, such as anti-allergic, antibiotic, anti-carcinogenic and anti-oxidative [3 - 7]. Numerous physiological and biochemical processes in the human may produce oxygen-centered free radicals and other reactive oxygen species. The over production of these components during the metabolism and other activities beyond can cause oxidative stress. This imbalance is developed in the organism is an important contributor to the pathophysiology of a variety of pathological conditions including cardiovascular dysfunction, atherosclerosis, inflammation, carcinogenesis, neurodegenerative disorders, aging, chronic degenerative diseases and inflammation [1 - 2] [8 - 9].

Antioxidants (AO) are vital substances which possess the ability to protect the body, cells and tissues from continuously threatened by the damage caused by free radical and reactive oxygen species which are produced during normal oxygen metabolism or are induced by exogenous damage [10]. The mechanisms and interferences with cellular functions are not fully understood, but one of the most important events seems to be the attenuation of the oxidative damage of a tissue can be indirectly, by enhancing natural defence of cell and/or directly by scavenging the free radical species [11]. These compounds are named in two categories which are the natural and synthetic which have some side effects [12]. Antioxidants components include carotenoids, vitamins, flavonoids and phenols that are microconstituents capable to prevent the destructive process caused by oxidative stress [13].

The phenolic compounds are secondary metabolites in plants and possess a wide and complex array of phytochemicals that exhibit several health beneficial activities such as antioxidant, antiinflammatory, antihepatotoxic, antitumoral and antimicrobial [14 - 16]. Phenolic phytochemicals are known to exhibit several health beneficial activities such as compounds with an antioxidant activity, including phenolic acids and flavonoids. Flavonoids are naturally occurring in plant and are though to have positive effects on human health and are the major group of free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action [17 - 18].

More than 4000 varieties of flavonoids have been identified many of which are responsible for the attractive colours of flowers, fruit and leaves [10]. Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity [19]. Natural antioxidants either in the form of raw extracts or their chemical constituents are very effective to prevent the destructive processes caused by oxidative stress [13]. The toxicity profile of medicinal plants have not been evaluated, it is generally accepted that medicines derived from plant products are safer than their synthetic counterparts [20] . Joshny et al., 2012 [21] have been described that medicinal plants which are a part of earth´s biodiversity, now a days the most important medicines are also based on more than 90 species of plants with distinguishable medicinal properties, among which one is *Bougainvillea glabra*.

An ornamental flowering plant from the genus of *Bougainvillea* and *Nyctaginaceae* family is widely distributed in Cuernavaca, Morelos and other states of Mexico, such as *B. xbuttiana*. Due to their pharmacological effects of this plant is widely used in traditional and modern medicine for preparation of cough, bronchitis, respiratory infection, gastritis hyperacidity, gastroduodenal ulcer, colic, fever, diarrhoea, injury, diabetes, and stomach ache and it is also used as expectorant [18]. We are the first group that described the antinociceptive and anti-inflammatory activities of *Bouganvillea xbuttiana* [22]. The current study aimed to investigate and compare the antioxidant activity, phenolic and flavonoids contents and proliferative activity in various ethanolic extracts from flowers of different colours of *Bouganvillea xbuttiana.*

MATERIAL AND METHODS

Chemicals, reagents and buffers

2,2´-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, quercetin, Folin-ciocalteu reagent, aluminum chloride, ferric chloride, fetal calf serum (FCS), RPMI-1640 medium were purchased from Sigma (St. Louis, MO, USA). All other reagents were analytical grades.

Extraction of *Bougainvillea xbuttiana*:

The aerial part (flowers) of the plant was collected from Cuernavaca (Mor. Mexico) in May 2010 and identified. A voucher specimen (23683) is deposited at the Herbarium HUMO, CIByC (UAEM). The extraction, fractionation and isolation of *Bougainvillea xbuttiana* extracts were performed as described (Patent MX/a/2011/813522). Briefly, the flowers were cleansed using water and dried at room temperature, powdered and extracted. The powdered plant material (500 g) was macerated with ethanol 50% for 72 hours at 25ºC. The extract was collected at room temperature, filtered and evaporated to dryness under reduced pressure in a rotary evaporator. The percentage yield was found to be 2.79 g. The ethanolic extract of *Bougainvillea xbuttiana* was referred as *Bxb* [22]. The ethanolic extracts used in this study were obtained from different colors of *Bougainvillea xbuttiana* named as: orange-1 (*BxbO1*); orange-2 (*BxbO2*); rose (*BxbR*); violet (*BxbV*) and white (*BxbW*).

Effect of Bxb extracts on cells

Viability assay: HeLa cells were seeded on 96-well plate at 1 X 10^5 cells/well, control and/or treated cells with different concentrations of *BxbO1; BxbO2; BxbR; BxbV and BxbW* extracts and incubated at 37°C in a 5% CO_2 atmosphere. After distinct times of incubation, the supernatants were removed and the remaining live cells assessed by fixing and staining with crystal violet 0.2% in 20% methanol. The percentage viability was determined by measured of absorbance in each well by reading at 620 nm in a microplate reader and calculated as follows: [(A_{sample}/ $A_{control}$) X 100]. *Vacuolation assay:* HeLa cells were maintained as described above. For the vacuolation assay the cells were incubated with the RPMI-1640 medium supplemented with 5% FCS and 1 mM/mL NH_4Cl and exposed to different amounts of *Bxb* extracts [23]. The plates were incubated for different time periods at 37°C with 5% CO_2. To detect the vacuoles, cell cultures were stained with 0.05% neutral red solution for 5 minutes. The cells were washed with PBS containing 0.2% BSA, 70% ethanol and 0.37% HCl. Absorbance was determined using microtiter reader plate at 540 nm. Vacuolating percentage was calculated as equation: [(A_{sample} – $A_{control}$/ $A_{control}$) X 100].

Determination of total phenolic contents (TPC)

The concentration of phenolics in different *Bxb* extracts colors was determined using the spectrophotometric method described by Singleton *et al.*, 1999 [24]. In brief, ethanolic extracts from different colors of *Bxb* in the concentration of 1 mg/ml were used in the analysis. The reaction mixtures were prepared by mixing 0.5 ml of dry extracts, 2.5 ml of 10% Folin-ciocalteu dissolved in water and 2.5 ml 7.5% $NaHCO_3$. For the prepare the blank 0.5 ml ethanol, 2,5 ml 10% Folin-ciocalteu reagent dissolved in water and 2.5 ml of 7.5% of $NaHCO_3$. Subsequently, the mixture was incubated at 4°C for 30 min and its absorbance was measured at 760 nm using spectrophometer. The TPC was expressed in terms of galic acid equivalents (mg of GA/g of extract) using the following equation based on the calibration curve: Y = 2.2144x + 0.962 R^2 = 0.9906 where x was the absorbance and Y was the mgQE/g.

Determination of total flavonoid content (TFC)

Total flavonoids contents were estimated in ethanolic extracts from distinct colors of *Bxb* with the method described by Zhishen *et al.*, 1999 [25]. Briefly, 0.5 ml of extract was added to 75 µl of 5% $NaNO_2$ solution. After 6 min., 150 µl of a 10% $AlCl_3.6H_2O$ solution was added and the mixture was allowed to stand another 5 min.. Then, 0.5 ml of 1 mol NaOH and 2.5 ml of distilled water was added. The solutions were mixed and its absorbance was determined at 510 nm using spectrophotometer. The experiments were carried out in triplicate. TFC was expressed as mgQE/g of extract, using the following equation based on the calibration curve: Y = 2.2224x + 0.982R^2 = 0.9917 where x was the absorbance and Y was the mgQE/g.

Antioxidant activity

DPPH Radical Scavenging Activity

DPPH radical scavenging of *Bxb* extracts was measured by 2,2´-diphenyl-1-picrylhydrazyl (DPPH) assay according to the method of Miliauskas *et al*., 2004 [26]. The stock solution was prepared by dissolving 24 mg of DPPH with 100 ml methanol and stored at 20°C until use. The working solution was obtained by diluting DPPH solution with methanol to attain an absorbance of about 0.98 ± 0.02 at 515 nm using the spectrophotometer. A 3 ml of this solution was mixed with 100 µl of the sample at various concentrations (2.9 to 940 µg). The reaction mixture was shaken will and incubated in water bath for 20 min. at 37°C, and the decrease in absorbance at 515 nm was determined. The control was prepared as above without any sample. Percentage inhibition was estimated using the following equation: % inhibition = [($A_{control}$ - A_{sample}/ $A_{control}$) X 100]. The IC_{50} values were concentration providing 50% inhibition of DPPH radicals and were calculated from the regression equation, developed by plotting concentration of the samples versus percentage inhibition of free radicals. Here, all test analysis was run in triplicate and average values were reported, quercetin was used as positive control.

The antioxidant activity was expressed as the antioxidant activity index (AAI) calculated as follows equation: AAI = final concentration of DPPH in the control sample µg.ml^{-1} / IC_{50} µg.ml^{-1}. Thus the AAI values was calculated considering the mass of DPPH and the mass of the tested compound in the reaction resulting in a constant for each compound independent of the concentration of DPPH and sample use. The AAI values were considered as poor, moderate, higher and strong antioxidant activity as described by Scherer and Godoy, 2009 [27].

Determination of ferric reducing ability of plasma (FRAP)

Antioxidant activity of different *Bxb* extracts colors was measuring colorimetrically using the ferric reducing ability of plasma assay described by Benzie and Szeto, 1999 [28]. FRAP assay was determined based on the reduction of Fe^{+3}-TPTZ to a blue colored Fe^{+}-TPTZ. In brief, the FRAP reagent was prepared fresh and was warmed to before using FRAP reagent (900 µl) was pipetted into test tubes.

A total of 30 µl of sample and 300 µl of distilled water was then added to the same test tubes, and incubated at for 30 min in the dark condition. Reading of the colored product was then taken at 595 nm. The standard curve was using iron (II) sulphate solution (0 – 2000 µM), and the reducing power were expressed as equivalents concentration (EC1). FRAP value was expressed in terms of mmol Fe^{+2} /g of sample using ferric chloride standard curve Y = 1.8075x – 0.2121, R^2 = 0.9901. All of measurement was taken in triplicate and the mean values were calculated.

STATISTICS

Statistical analyzes was carried out using ANOVA with significance level set at $p < 0.05$. Differences among means were evaluated using a statistical program. All experiments were carried out in triplicate and repeated at least twice.

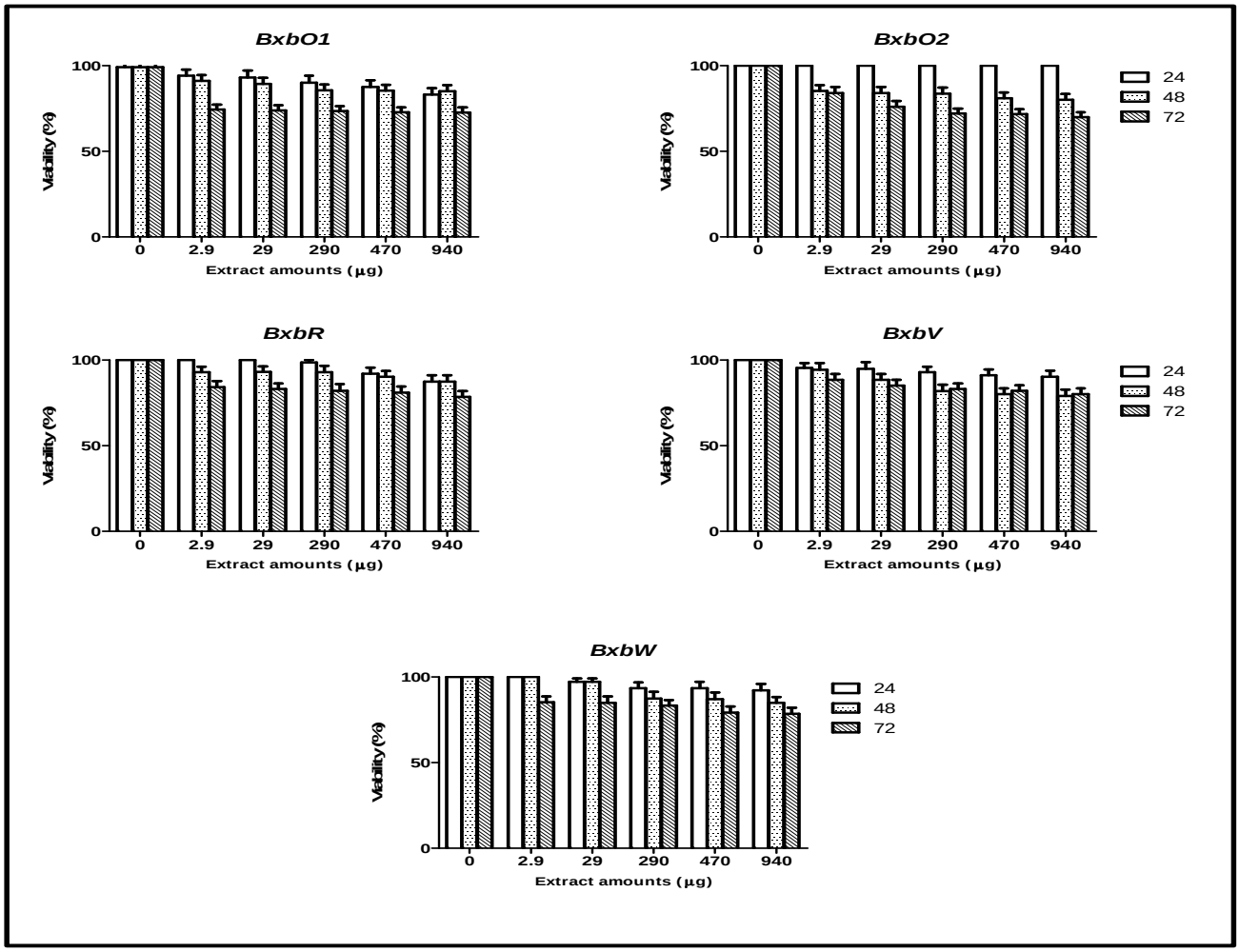

Figure 1: Viability percentage.

Fig. 1: Viability percentage

Table 1: The scavenging activity (IC_{50} values) on DPPH radicals and anti-oxidant activity index of *B.xbuttiana* extracts

Extracts	IC_{50} (µg/ml)	AAI
BxbO1	830	0.332
BxbO2	108	1.57
BxbR	235	1.17
BxbV	750	0.256
BxbW	429	0.5

RESULTS

Effect of *Bxb* on HeLa cells.

To determine the effect *Bxb* on HeLa cells that were exposed to different concentrations of *Bxb* extracts from flowers of distinct colors and incubated under the same conditions for 24, 48 and 72 hs. The effects of *Bxb* extracts were determined by measuring viability percentage and vacuoles formation.

Viability: To determine the viability percentage the HeLa cells were treated as described above. The cells were exposed during different periods of time to *Bxb* extracts showed cytotoxic effect that was dose and color-dependent manner (Fig. 1). The lowest viability percentage was observed in cultures of HeLa cells treated with 940 µg/ml of all color *Bxb* extracts for 72 hours. The viability percentage at this period was 80%, 78.6%, 78.5%, 72.8% and 70% for *BxbV*, *BxbW*, *BxbR*, *BxbO1* and *BxbO2*, respectively (Fig. 1). The more high cytotoxicity percentage was observed in HeLa cells that were exposed during 72 hours to 940 µg/mL of *BxbO2* and *BxbO1* extracts, 30% and 27.4%, respectively (Fig. 2). Vacuoles formation: In order to determine the vacuolation in HeLa cells the assay were carry out as described above. The extent of vacuolation was quantified over a different times using neutral red assay. HeLa cells were exposed to 940 µg of *Bxb* extracts for distinct periods of time. The percentage of vacuoles formation in HeLa cells is shown in Figure 3. For culture cells treated with *BxbO1* extract no vacuoles was observed in all periods here studied. The highest percentage of vacuoles formation was in culture cells treated with 940 µg/mL of extract for 48 hours. At this period and concentration used the percentages of vacuoles formation are 32.85%, 23.07%, 21.18% and 6.94%, for *BxbR*, *BxbW*, *BxbO2* and *BxbV*, respectively (Fig. 3).

Antioxidant activity

DPPH radical scavenging

To determine the antiradical activities of *Bxb* extracts were assessed using 2,2´-diphenyl-1-picrylhydrazyl radical scavenging assay. This method depends on the reduction of purple DPPH to a yellow colored diphenyl-picrylhydrazine and the remaining DPPH. The results of the assay for antioxidant activity are shown in Figure 4.

The examination of antioxidant activity of extracts from *Bxb* showed values varied from 8.72 to 81.03%.

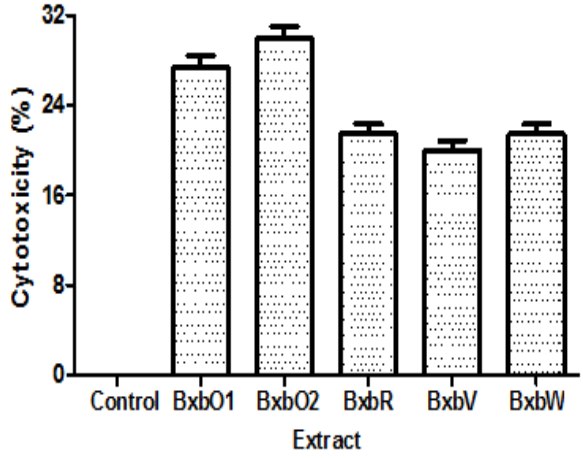

Fig. 2: Cytotoxicity percentage.

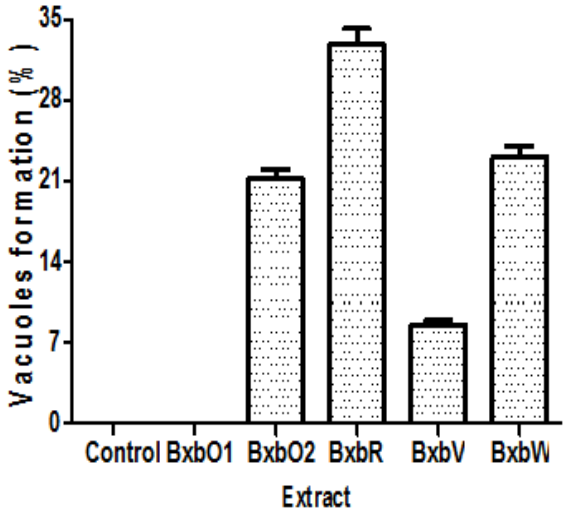

Fig. 3: Vacuoles formulation

The largest capacity to neutralize DPPH radicals was found for *BxbO2* and *BxbR*, which neutralized 50% of free radicals at the concentration of 108 µg/mL and 235 µg/mL, respectively (Table 1). A moderate activity was found for *BxbW* extract, which neutralized 50% of free radicals at the concentration 429 µg/mL, respectively (Table 1). Due to low activity of *BxbV* and *BxbO1* extracts, which were capable to neutralize 50% of free radicals at the concentration 750 µg/mL and 830 µg/mL, respectively (Table 1). The antioxidant activity index for extracts obtained from extracts of *BxbO2* and *BxbR* was significantly higher when compared with those obtained for *BxbW, BxbV* and *BxbO1* extracts ($p < 0.001$) (Table 1). Poor antioxidant activity when AAI values are < 0.5 observed in extracts of *BxbO1* and *BxbV*. Moderate antioxidant activity when AAI values are between 0.5 and 1.0 obtained in the *BxbW* extract. Strong antioxidant activity when AAI values are between 1.0 and 2.0 showed in *BxbO2* and *BxbR* extracts.

Ferric reducing ability of plasma values

Another method as the reduction power assay was used to confirm the *Bxb* extracts antioxidant activity. The reduction capacity of the ethanolic Bxb extracts is indicated in Figure 5. The FRAP values were 1,95 $mmolFe^{+2}$ /g, 2.13 $mmolFe^{+2}$ /g, 2.17 $mmolFe^{+2}$ /g, and 2,28 $mmolFe^{+2}$ /g for *BxbO1*, *BxbV*, *BxbW* and *BxbO2* and *BxbR*, respectively (Fig. 5). The results obtained in this study are confirmed the antioxidant activity for these *Bxb* extracts and it is clear that existence of discrete differences between the extracts in a capacity of reducing power.

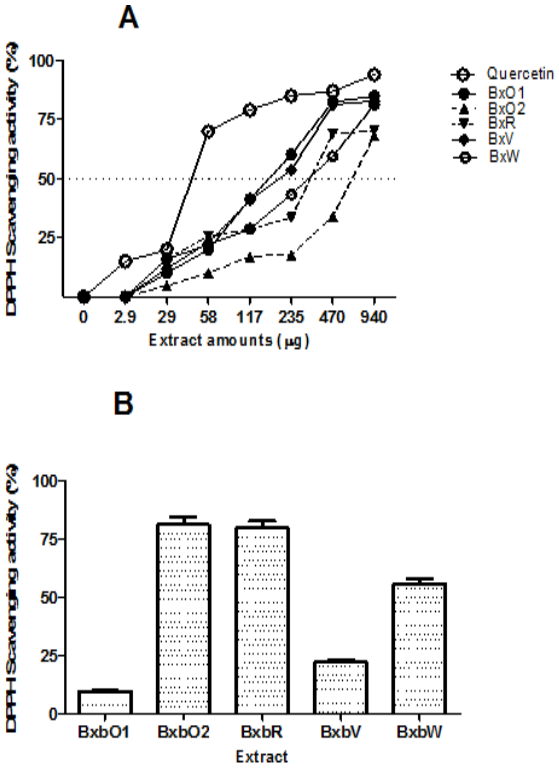

Fig. 4: DPPH Scavanging activity.

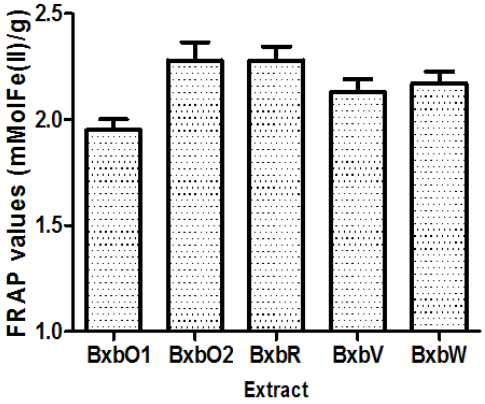

Fig. 5: FRAP Values.

Total phenolic content

To determine the total phenolic contents in the examined ethanolic extracts using the Folin-ciocalteu´s reagent. The TPC was expressed in terms of galic acid equivalents (mg of GA/g of extract) using the following equation based on the calibration curve: $Y = 2.2144x + 0.962$ $R^2 = 0.9906$ where x was the absorbance and Y was the mgQE/g. The values obtained for the concentrations of total phenolic content are expressed as mg of GA/g of extract (Fig. 7).

The highest phenolic content was measured in *BxbO2* extract with values of 28.89 mgGA/g and the lowest TPC values was observed in *BxbO1* extract with 14.68 mgGA/g (Fig. 6). For the extracts from *BxbR*, *BxbW* and *BxbV* the phenolic contents were 23.67, 22.16 and 20.71 mgGA/g, respectively (Fig. 6).

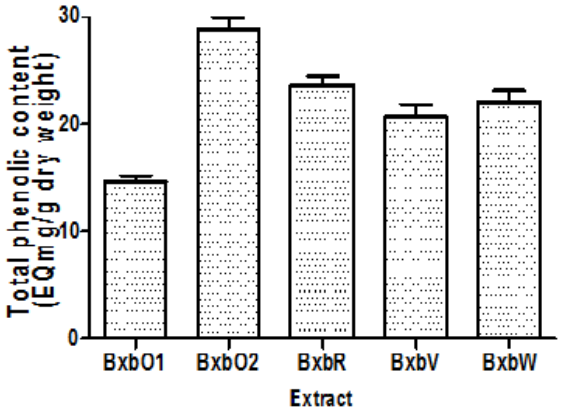

Fig. 6: Total Phenolic constant

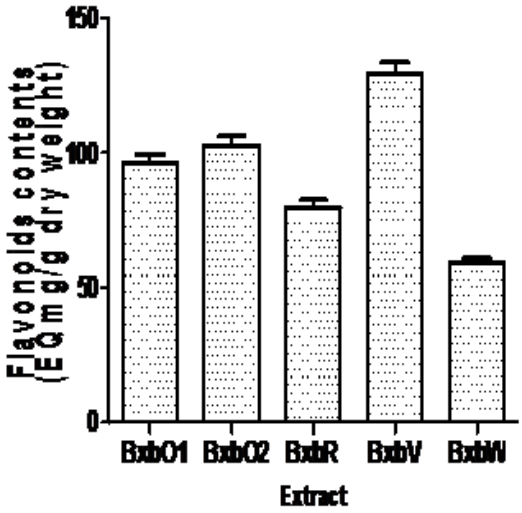

Fig. 7: Total flavonoids.

Flavonoids contents

To determine the concentration of flavonoids in different *Bxb* extracts was assayed using spectrophotometric method with aluminum chloride. The content of flavonoids was expressed in terms of mgQE/g of extract (Fig. 7). The flavonoids concentration in *Bxb* extracts ranged from 59.13 to 129.38 mg/g. The highest value of flavonoids in *BxbV* was 129.38 mgQE/g. The concentration of flavonoids observed for the others extracts was 102.7, 96.9, 79.8 and 59.1 mg/QE/g for *BxbO2, BxbO1, BxbR* and *BxbW,* respectively (Fig. 7).

DISCUSSION

In the present study ethanolic extract of *Bxb* from flowers of different colors native from Mexico was compared for its effects on cells and antioxidant activity.

To assess the toxicity of the extracts towards is routinely and widely used the method of HeLa cells viability and vacuoles formation. In the literature are described that plant extracts contains a higher concentrations of bioactive compounds and also several compounds which show cytotoxic activity. In this study the exposition of HeLa cells to *B. xbuttiana* extracts with 940 µg/mL caused lysis and morphological changes. We also observed that *B. xbuttiana* extracts mediated vacuole formation in HeLa cells and in a concentration-dependent manner. In this study we observed that the presence of large vacuoles and membrane projections were not damaging. The cells presented plasma membrane integrity and well preserved mitochondria profiles, characteristics of good cell viability.

To the best of our knowledge the present study also evaluated the ability of the ethanolic extracts from *Bxb* to act as antioxidant agents. Several evaluation protocols have been used to determine the antioxidant activity to allow rapid screening of substances. There is great of methods for determination of antioxidant capacity of extracts and beverages based on different principles. The most widely used procedures for measurement of antioxidant activity capacity are the DPPH radical scavenging analysis is one of the best-known, accurate, and frequently employed methods for evaluating antioxidant activity [29]. It is a stable free radical because of its spare electron delocalization over the whole molecule. The degree of color change is proportional to the concentration and potency of the antioxidants. The DPPH scavenging also made a proportionate decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound under test [30]. The experimental data revealed that all extracts of *Bxb* have effects of scavenging free radicals and a color dependent relationship in the DPPH radical scavenging activity. In this study the IC_{50} values ranged from 108 to 830 µg/mL for DPPH radical scavenging for the aerial part of *B. xbuttiana* extracts. Low IC_{50} value indicates strong ability of the extracts to act as DPPH scavenging observed for *BxbO2* and *BxbR* extracts, a high IC_{50} values indicates low scavenging activity was observed in *BxbO1* and *BxbV*. IC_{50} values of *BxbO2* and *BxbR* were found to be significantly better than other extracts ($p < 0.001$). In this study, it was considered that extracts showed poor antioxidant activity when AAI < 0.5 observed for *BxbV* and *BxbO1* extracts. Moderate antioxidant activity when AAI between 0.5 and 1.0, for *BxbW* extracts, strong antioxidant when AAI between 1.0 and 2.0 for *BxbO2* and *BxbR* extracts. These values are in according to described by Schere and Godoy, 2009 [27]. The ranking order for the antioxidant activity index were *BxbO2* > *BxbR* > *BxbW* > *BxbV* > *BxbO1*.

The reducing power assay the yellow colors of the test solution changes to green depending in the reduction of the Fe^{+3}/ferricyanide complex to the ferrous form. Therefore, Fe^{+2} can be quantifying by absorbance measurement at 700 nm [31]. The higher the absorbance of the reaction mixture indicates an increase in reducing power. The presence of reductants in *Bxb* extracts caused the reduction of the Fe^{+3} into Fe^{+2}. Reducing power was found to be in order *BxbO2* = *BxbR* > *BxbW* > *BxbV* > *BxbO1*. It has been reported that the reducing power of substances is probably because of their hydrogen-donating ability [32].

The *BxbO2*, *BxbR* and *BxbW* extracts might therefore, contain high amount of reductors than that observed in *BxbO1* and *BxbV* extracts. Hence, ethanolic extracts of *B.xbuttiana* may act as electron donors and could react with free radicals to convert them into more stable products and them terminate the free radical chain reactions. Phenolic compounds are considered secondary metabolities and these phytochemical compounds derived from phenylalanine and tyrosine occur ubiquitously in plants and are diversified [33]. The TPC assay is another that is commonly used in conjunction with either of both of the DPPH and FRAP assays, again presumably with the aim of increasing the information data base on *B. xbuttiana* extract. Phenolic compounds may be responsible for the possible antioxidant activity of many plants because their hydroxyl groups confer scavenging ability [34]. The results presented in this study indicate that high antioxidant activity is associated with a high phenolics content, a finding reported previously many times [35 - 37]. Some flavonoids effects include free radical scavenging inhibition of hydrolytic and oxidative implicated in several diseases [17]. A variety of *in vivo* and *in vitro* experiments have shown that selected flavonoids possess antibacterial, antiviral, anti-inflammatory, anticancer and anti-allergic activities [17, 38]. The total flavonoids contents equivalents were used for the measurement of *B. xbuttiana* extracts. In this study, the ranking order of the total flavonoids contents was *BxbV* > *BxbO2* > *BxbO1* > *BxbR* > *BxbW*. Our results suggested that TPC and TFC may be the major contributors to the antioxidant activity of the *B. xbuttiana* extracts.

CONCLUSION

To assess the antioxidant activity of sample, variety of methods must be used in parallel with two methods used the *B. xbuttiana* extracts presented poor, moderate and strong antioxidant activity the

manner color-dependent. Our results suggested that phenolic and flavonoids may be the major contributors for the antioxidant activity. In the present study analysis of free radical scavenging activity and total phenolic and flavonoids content from the *B. xbuttiana* flowers can be the potent source of natural antioxidants.

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Competing Interest

The authors declare that they have no competing interests

REFERENCES

1. Arct J, Pytkowska K. Flavonoids as components of biologically active cosmeceuticals. Clin. Dermatol.2008: 26: 347-357.
2. Soobrattee MA, Neergheen VS, A. Luximon-Ramma, Aruoma OI, Bahorun T, Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. Mutat. Res.2005: 579: 200-213.
3. Borneo R, Leon EA, Aguirre A, Ribotta P, Cantero jj. Antioxidant
4. capacity of medicinal plants from the Province of Cordoba (Argentina) and their *in vitro* testing in model food system. Food Chem. 2008: 112: 664-670.
5. Hoffer A. ABC of Natural Nutrition for children. Quarry Press Inc., Kingston (ON), p. 57. 1999.
6. Null G. Garrz Null's Ultimate Lifetime Diet, Broodway Books, New York, p. 87, 2000.
7. Parker L, Colman C. The Antioxidant Miracle, John Wiley and Sons, Inc., New York, p. 126, 2000.
8. Stankovic MS, Curcic MG, Zizic JB, Topuzovic MD, Solujic SR, Markovic SD. *Teucrium* plant species as natural sources of novel anticancer compounds: antiproliferative, proapoptotic and antioxidant properties. Int J Mol Sci.2011: 12(7):4190-205.
9. Aruoma OI. Free radicals, oxidative stress and antioxidants in human health and disease. JAOCS vol. 75, N.2, 1998.
10. Martinez-Valverde I, Periago MJ, Ros G. Significado nutricional de los componentes fenólicos de la dieta. Archivos Latinoamericanos de Nutrición.2000: 50: 5-18.
11. de Groot H, Rauen U. Tissue injury by reactive oxygen species and the protective effects of flavonoids. Fundam Clin Pharmacol. 1998: 12:249–55.
12. Verma AR, Vijayakumar M, Mathela CS, Rao CV. *In vitro* and *in vivo* antioxidant properties of different fractions of *Moringa oleifera* leaves. Food and Chemical Toxicology. 2009: 47(9): 2196-2201.
13. Branien AL. Toxicology and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. J Am Oil Chem Soc. 1975: 52(2):59-63.
14. Zengin G, Guler G, Cakmar Y. Antioxidant capacity and fatty acid profile of Centaurea kotschyi (Boiss. & Heldr.) Hayek var. Persica (Boiss.). Wagenitz from Turkey. *Grasas y aceites.* 2011: 62(1): 90-95.
15. Hertog MGL. Flavonoid intake and long-term risk of coronary heart disease and cancer in the 7 countries study. Arch.Intern.Med., 1985: 155:1184-1195.
16. Middleton E, Kandaswami C, Theoharides J. The effects of flavonoids on mammalian cells implications for inflammations, heart disease, and cancer. Pharmacol. Reviews, 2000: 52: 673-751.
17. Rice-Evans CA, Miller NJ, Paganga G. 1996. Antioxidant properties of phenolic compounds. Trends in Plant Science, 4, R., Khan, R.,1996: 304-309.
18. Di Carlo G, Mascolo N, Izzo AA, Capasso F: Flavonoids: old and new aspects of a class of natural therapeutic drugs. Life Sci. 1999: 65:337-353.
19. Frankel EN. 2007. Antioxidants in Food and Biology, Facts and Fiction. The Oily Press, Bridgwater, England, 2007: 254 pp, 2007.
20. Gryglewski RJ, Koburt R, Robak J.On the mechanism of antithrombotic action of flavonoids. Biochemical Pharmacology.1987: 36: 317-321.
21. Saeed N, Khan MR, Shabbir M. Antioxidant activity, phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla L*. BMC Complementary & Alternative Medicine.2012: 12: 221-233.
22. Josnhy J, Ramya Devi D, Vedha Hari BN. Phytochemical and *in vitro* anthelmintic activity of hydro alcoholic extract of *Bougainvillea glabra*. International Journal of Pharmacy and Pharmaceutical Sciences.2012: 4(2):1-3.
23. Alvarez Perez Gil, AL, Barbosa Navarro L, Patiño Vera M, Petricevich VL. Anti-inflammatory and antinociceptive activities of the ethanolic extract of *Bougainvillea xbuttiana* J Ethnopharmacol. 2012:144(3):712-9.
24. Cover TL, Puryear W, Perez-Perez GI, Blaser MJ. Effect of urease on HeLa cell vacuolation induced by Helicobacter pylori cytotoxin. Infect Immun. 1991: 59(4):1264-70.
25. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 1999: 299: 152-178.
26. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry. 1999: 64: 555–559.
27. Miliauskas G, Venskutonis P, van Beek T. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem. 2004: 85: 231-237.
28. Scherer R, Godoy HT. Antioxidant activity index (AAI) by the 2,2-diphenyl-1-picrylhydrazyl method. Food Chemistry. 2009: 112(3): 654-658.
29. Benzie IFF, Szeto YT. Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. Journal of Agricultural and Food Chemistry, 1999: 47(2): 633-636
30. Zhou K, Yu L. Antioxidant properties of bran extracts from Trego wheat grown at different locations. J. Agric. Food Chem. 2004: 10; 52(5): 1112-1117.
31. Krishnaiah D, Sarbatly R, Nithyanandam RR: A review of the antioxidant potential of medicinal plant species. Food Bioprod Process.2011: 89:217-233.
32. Saha AK, Rahman MR, Shahriar M, Azad NA, Das S. Screening of six ayuvedic medicinal plant extracts for antioxidant and cytotoxic activity. J. Pharmacog. Phytoche,2013: 2:181-188.
33. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural and Food Chemistry.1992: 40, 6: 945-948.
34. Naczk M, Shahidi F. Extraction and analysis of phenolics in food. J. Chromatogr. A. 2004: 1054: 95-111.
35. Elmastas M, Isildak O, Turkekul I, Temur N. Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. J. Food Compos Anal.2007: 20(3-4): 337-45.
36. Dudonné S, Vitrac X, Coutiere P, Woillez M, Merillon .-M. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. J Agric Food Chem. 2009: 57(5):1768-74.
37. Qader SW, Abdulla MA, Chua LS, Najim N, Mat Zain M, Hamdan S. Antioxidant, total phenolic content and cytotoxicity evaluation of selected Malasyan Plants. Molecules.2011: 16: 3433-3443.
38. Xu C, Zhang Y, Cao L, Lu J. Phenolic compounds and antioxidant properties of different grape cultivars grown in China. Foof Chem. 2010: 119: 1557-1565.
39. Montoro P, Braca A, Pizza C, De Tommasi N. Structure-antioxidant activity relationships of flavonoids isolated from different plant species. Food Chem. 2005: 92:349-355.