PAPER

ANTIOXIDANT AND ANTI-INFLAMMATORY CAPACITIES OF PEPPER TISSUES

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ABSTRACT

The objective of this study was to investigate the antioxidant and anti-inflammatory activities of five pepper varieties tissues. Green Bell peppers had the highest total antioxidant contents; while Red Chilli variety had the lowest antioxidant activities (ABTS was 3.89 μmol TE/g fw, DPPH was 2.82 μmol TE/g fw and FRAP was 16.95 μmol TE/g fw). The methanolic extracts of different peppers showed strong but different anti-inflammatory activity values (8.22 μg/ml - 9.52 μg). Yellow Bell, Red and Green Chilli had the highest anti-inflammatory activity followed by Green and Red Bell extracts, respectively. The results suggest that these varieties of pepper could contribute as sources of important antioxidant and anti-inflammatory related to the oxidative stress and inflammation prevention.

Keywords: pepper, antioxidant, anti-inflammatory, cell line

1. INTRODUCTION

The interest in natural food full of antioxidants and their therapeutic properties have recently increased dynamically. Pepper fruit belongs to the genus Capsicum, *Solanaceae* family with of more than 200 varieties (ZIMMER *et al.*, 2012; ALLEMAND *et al*., 2016). Pepper has spread of names reckoning on location and type; and the most common pepper names are chili, bell, green and red or just pepper (SUNG *et al.*, 2016). Pepper is considered an excellent source of bioactive nutrients (AMINIFARD *et al.*, 2012). The nutritive composition of peppers depends mainly on the several factors, including cultivar, agricultural practice (organic or conventional), maturity and storage conditions (NIMMAKAYALA *et al.*, 2016). Pepper fruits are popular due to their characteristic as flavor, texture, firmness and bright colors (SILVA *et al*., 2014a). In addition, peppers consumption is recommended due to the positive bioactive compounds impact on health, such as minerals, vitamins and antioxidant compounds (SILVA *et al*., 2013; SILVA *et al*., 2014b). The phytochemicals in pepper fruits have been reported to possess many pharmacological and biochemical properties, such as anti-allergic, anti-carcinogenic, antioxidant and anti-inflammatory activities (ROKAYYA *et al.*, 2019). They can be eaten fresh, pickled, smoked, dried, or in sauces (ALVAREZ-PARRILLA *et al.*, 2012). In fact, in Chinese medicine, pepper has been used for of stomach aches, arthritis, rheumatism, skin rashes, dog/snake bites and flesh wounds treatments (KIM *et al.*, 2016). The antioxidant properties were tested in several studies by using different approaches (LONKAR and DEDON, *et al.*, 2011). Free radical scavenging activity (DPPH), ferric reducing antioxidant power (FRAP), and trolox equivalent antioxidant capacity (ABTS) assays are the three most frequently used for measuring the antioxidant activities (MAGALHAES *et al.*, 2011).
Oxidative stress plays an essential role in cardiovascular diseases and pathogenesis cancer (MONTECUCCO *et al.*, 2011). The redox stress triggers the activation of immune cells which release pro-inflammatory cytokines, reactive nitrogen and oxygen species causing pathological pathways and physiological imbalances (LONKAR *et al*., 2011).
The present study, therefore aims to determine the total antioxidant, flavonoid and phenol, measure the bioactive activities such as (FRAP), (ABTS), (DPPH), NO production and cell viability (MTT).

2. MATERIALS AND METHODS

2.1. Chemicals and cells

ABTS (2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP, Quercetin, Folin-Ciocalteau, Trolox reagents and ascorbic acid, were from (Sigma, Louis, MO, USA). Dulbecco's modified eagle medium (DMEM) and LPS were purchased from Sigma Inc. (St. Louis, MO, USA). The murine machrophage cell line, RAW 264.7 was purchased institute of biological sciences (Shanghai, China).

2.2. Sample preparation

Five different pepper varieties purchased from Shenyang city, China: Yellow Bell, Red Bell, Green Bell, Red Chilli and Green Chilli, respectively. Bell type (Yellow, Red and Green) and Chilli type (Red and Green). Yellow, Red and Green are Bell type from flowering plants genus in the nightshade family *(Solanaceae);* pepper with thick skin fruits

(approximately 112–217 g in weight); Red and Green Chilli are the fruits of genus *Capsicum* plants which are nightshade family *(Solanaceae)* members. Chilli peppers are varieties with cone shape and medium size (61-91 g in weight). Peppers were purchased in a local supermarket at commercial maturity. All the pepper varieties were cleaned and cut tissues into cubic of 10 . 10 . 10 mm^3 before processing. Freeze-dried (FD) treatment was operated 2h at -80°C then put in freeze drying machine (ALPHA 1-4 LSC, Germany) at -50°C and 0.04 atm for 48h. Tissues were grounded to powder, packed in N_2-vacuumed amber bottles and stored at -80°C until use.

2.3. Antioxidant extraction

Pepper powder (1.5 g) of each sample was extracted with 10 ml of 80% methanol, by stirring and sonicating for 20 min. The extracts were centrifuged at ~3000g for 20 min, the supernatant was stored at 4°C.

2.3.1. Total antioxidant, phenolic and flavonoid content determination

The total antioxidant capacity was determined by using ascorbic acid as a standard. The results were expressed as μg of ascorbic acid equivalent (AAE) per mg (PARASAD *et al*., 2013). The flavonoid content was determined on triplicate aliquots of the homogenous pepper extract (ILAHY *et al.*, 2011). Thirty-microliter aliquots of the extract were used for flavonoid determination. Samples were diluted with 90 μl methanol, 6 μl of 10% aluminum chloride, 6 μl of 1mol/l potassium acetate were added and finally 170 μL of methanol was added. The absorbance was done at 415 nm after 30 min. Quercetin was used as a standard for calculating the flavonoid content (mg Qe/g of fw).

2.3.2. Antioxidant activity determination (ABTS), (DPPH) and (FRAP) assay

Antioxidant activity was measured using the $ABTS^{\cdot}$ decoloration method using radical $ABTS^{\cdot}$ (2,2-azino-di-(3-ethylbenzothialozine-sulphonic acid) (KAUR *et al.*, 2013). DPPH assay was to evaluate ability of antioxidants toward the stable radical DPPH. A 0.2 ml aliquot of a 0.0062 mM of DPPH solution, in 20 ml methanol (95%) was added to 0.04 ml of each extract and shaken vigorously (SHUMAILA *et al.*, 2013). FRAP was operated according to the procedure (YOUNG *et al.*, 2013). The FRAP reagent included 300 (mM) acetate buffer, pH 3.6, 10 (mM) TPTZ in 40 (mM) HCl and 20 (mM) $FeCl_3$ in the ratio 10:1:1. Reduction of the ferric-tripyridyltriazine to the ferrous complex, which was measured at 593 nm. Results were expressed at μmol TE/g fw.

2.4. Anti-inflammatory activity

2.4.1. Anti-inflammatory extraction

Extracts were prepared by homogenization of 4 g of freeze-dried sample in 10 ml of 80% methanol, using an Ultra Turrax Digital Homogeniser T-25 (Ika Werke GMBH & Co., Staufen, Germany). Further, supernatants were combined and filtered using Whatman No. 1 paper. The concentrate was evaporated under vacuum to dryness. Finally, the extract residue was dissolved in (DMSO) dimethyl sulfoxide solution to give a final concentration of 20 mg/ml.

2.4.2. Cell viability assay

Mitochondrial respiration was determined by a mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Briefly, treated cells ($1x10^5$ cells/ml) were incubated with 5 mg/ml (MTT) in 96-well plates for 4h and solubilized in DMSO (150 µl/well). The extent of the reduction of MTT within the cells were measured at 490 nm (ALET *et al.*, 2015).

2.4.3. NO production measurement

For NO production determination, the amount of NO_2 in the supernatant of the media was measured by the Griess method (SURESH and PRIYA, *et al.*, 2015). Cells were incubated for 24h, after which the cell culture medium (0.2 ml) were added to aqueous extract of pepper varieties containing the Griess reagents (1%) sulfanilamide, (0.1%) naphthyl ethylene diamine dihydrochloride in (5%) H_3PO_4. The NO production was then determined based on the absorbance at 540 nm.

2.5. Statistical analysis

Data from replications of all varieties were subjected to a variance analysis (ANOVA) using SPSS (16.0.). Significant difference between the means was determined by Duncan's New Multiple Range Test ($p<0.05$). The correlation between the studied parameters was determined by (PCA) using XLSTAT software.

3. RESULTS AND DISCUSSION

3.1. Total antioxidant, phenol and flavonoid contents

In our study, all the extracts exhibited high total antioxidant activity values from 9.52 µg AAE/mg fw in Green Chilli to 12.70 µg AAE/mg fw in Green Bell (Table 1).

Table 1. Total antioxidant, phenol and flavonoid contents of selected pepper tissue varieties.

	Total Antioxidant µg AAE/mg	Total Phenol mM (TEAC)	Total Flavonid mg Qe/g
Yellow Bell	12.68±0.15^a	20.53±0.64^a	89.85±7.45^d
Red Bell	11.57±0.60^b	21.09±0.93^a	141.67±10.12^b
Green Bell	12.70±0.57^a	19.91±2.14^a	113.60±7.96^c
Red Chilli	10.21±0.18^c	20.45±1.71^a	75.85±4.20^d
Green Chilli	9.52±0.10^c	20.67±1.63^a	163.95±8.35^a

Values are the average of three individual samples each analyzed in triplicate ±standard deviation. Different uppercase superscript letters respectively indicate significant difference ($p < 0.05$) analyzed by Duncan's multiple-range test.

The antioxidant activity can be attributed to flavonoids and polyphenolic compounds found in it (KIM *et al.*, 2016). Total phenol contents ranged from 19.91 mM (TEAC) in Green Bell to 21.09 mM (TEAC) in Red Bell. Red and Green Chilli total phenol had similar values, 20.45 mM (TEAC) and 20.67 mM (TEAC), respectively. Flavonoid contents ranged from 75.85 mg QE/g fw to 163.95 mg QE/g fw, Green Chilli peppers had the highest flavonoid contents followed by Red Bell and Green Bell. Red Chilli and Yellow Bell varieties had lower flavonoid contents 75.85 mg QE/g fw and 89.85 mg QE/g fw, respectively than the other varieties (113.60 - 163.95 mg QE/g fw).

3.2. Antioxidant activity (ABTS, DPPH and FRAP)

Antioxidant activity results of pepper varieties were expressed as μmol TE/g fw in (Fig. 1). The antioxidant activity measured by ABTS· assay was between 3.89 μmol TE/g fw for Red Chilli and 5.18 μmol TE/g fw for Yellow Bell. The results obtained by DPPH assay were between 2.82 μmol TE/g fw for Red Chilli and 43.29 μmol TE/g fw for Green Chilli. The values of pepper varieties obtained by FRAP assay were between 16.15 μmol TE/ g fw for Red Chilli and 46.36 μmol TE/ g fw for Green Bell. Similar results of antioxidant activity using DPPH and FRAP assays have been reported (RAHIM and MAT, 2012). However, the antioxidant capacity also depends on many other factors including environmental conditions, genetics, post-harvest storage conditions, production techniques used, ext. (ANNA *et al.*, 2014).

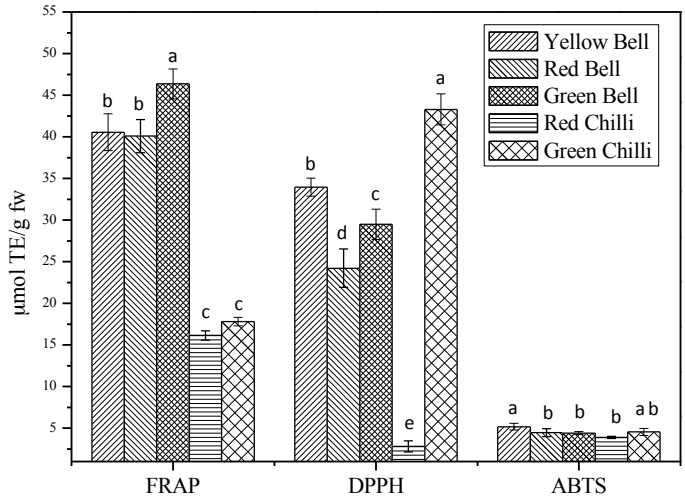

Figure 1. Antioxidant activities (ABTS, DPPH and FRAP) contents were expressed as (μmol TE/g fw).

3.3. Anti-inflammatory activity

The cytotoxicities of pepper extracts in LPS-induced macrophages were evaluated in a range of 0–200 μg extract/ml using MTT reduction assay after 24h (Fig. 2). Therefore, results indicated that the different concentration ranges of used in this study to treat the cells did not exert any cytotoxic effect. Analysis of NO production revealed that placing unstimulated RAW 264.7 cells in culture medium for 24h produced a basal amount of nitrite. When the cells were incubated with extracts from these varieties after treatment

with LPS for 24h, the medium concentration of nitrite increased markedly. Excessive production of NO in macrophages represents a probably noxious result, if not counteracted will cause the onset or progression of the many sickness pathologies (HAMIDREZA *et al.,* 2017). Significant concentration dependent inhibition was detected when cells were cotreated with LPS and various concentrations of the five variety extracts (Fig. 3).

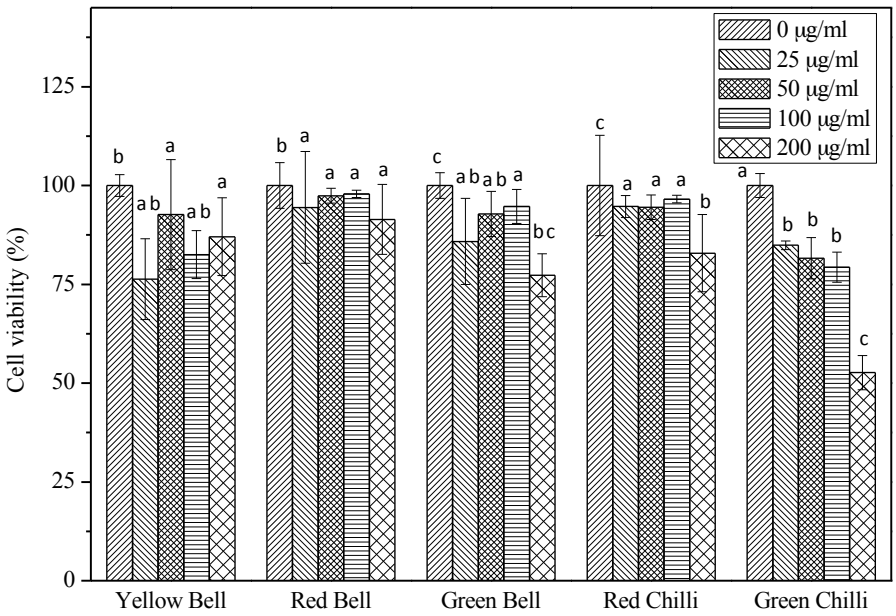

Figure 2. Cell viability.

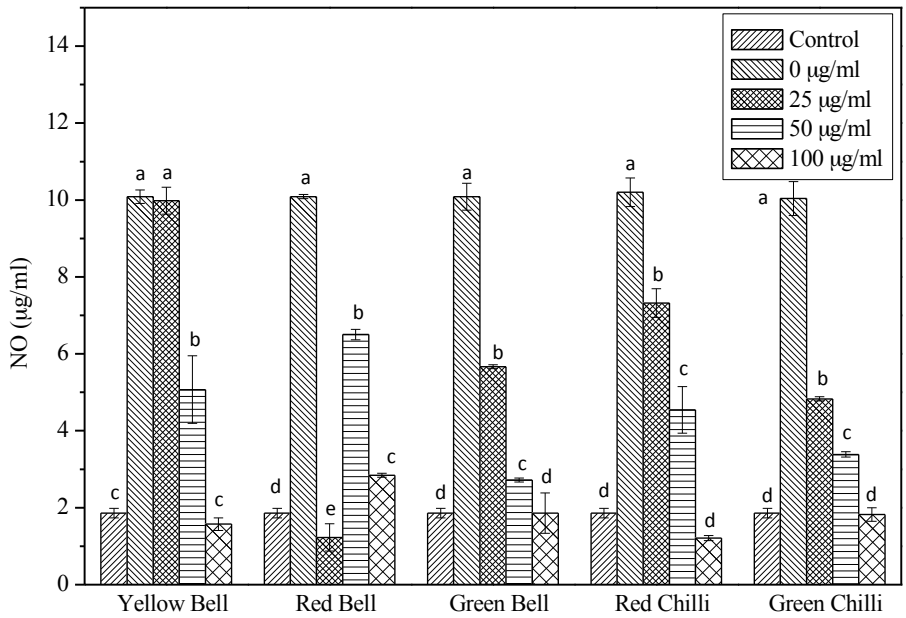

Figure 3. NO production by RAW 264.7 cells.

Pepper extracts induced a significant ($P<0.05$) dose-dependent suppression of NO production. All tested extracts showed high anti-inflammatory value in a range 0-100 μg concentration where Red, Green Chilli and Yellow Bell extracts were the best varieties followed by Green and Red Bell varieties. These results indicated that pepper had a noticeable effect on scavenging free radicals. NO production value grew equally in a dose dependant manner in all varieties except in Red Bell variety. A different reaction course was found for Red Bell. Red Bell showed an inverse relationship between the anti-inflammatory and the dose dependant manner at 25 μg/ml.

3.4. Correlation between antioxidant and anti-inflammatory activities

The analysis expressed that Green chili produced the lowest antioxidant level 9.52 μg AAE/mg fw with a little high anti-inflammatory activity 8.22 μg/ml (Fig. 4). Yellow Bell 12.68 μg AAE/mg fw and Green Bell 12.70 μg AAE/mg fw produced the highest antioxidant and high anti-inflammatory activity values 8.51 μg/ml and 8.23 μg/ml fw, respectively.

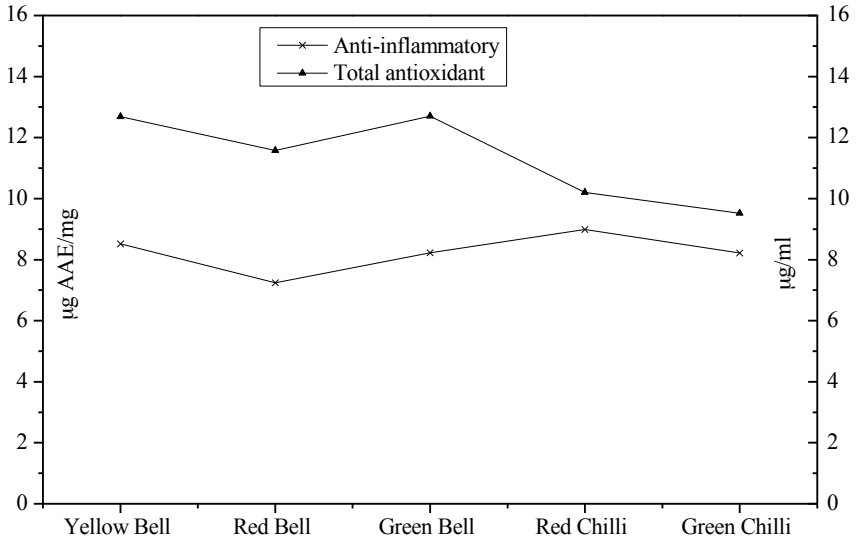

Figure 4. Correlation between antioxidant and anti-inflammatory activities.

The highest inflammatory activity level in Red Bell 7.25 μg/ml produced high antioxidant level 11.57 μg AAE/mg fw. Red Chilli produced a little high antioxidant and anti-inflammatory activity level 10.21 μg AAE/mg fw and 8.99 μg/ml, respectively.

3.5. Principal component analysis

Antioxidant and anti-inflammatory activities measurements had been submitted to (PCA) to presence of five subspecies of peppers, as seen in Table 2. The structuring accessions showed 70.38% of total variation. Axes were retained because they expressed 36.44% (axes 1), 33.94% (axes 2). Axes 2 was made positively by (DPPH), NO production, total phenol and flavonoid. The inertia was made negative by cell viability. Data projection on plans as

outlined by inertia axes of PCA from pepper samples showed vital significant variations between varieties. The (Figs. 5 and 6) present the plots of the scores/the correlation loadings respectively. In fact, once applying principal component analysis, it appeared that there was a discriminate structure. Yellow Bell and Green Bell were grouped together. As for Red Bell, Red and Green Chilli were individualized.

Table 2. Discriminate variables factors of principal components analysis.

	F1	F2
Proper Value	2.92	2.72
Variability (%)	36.44	33.94
Cumulative (%)	36.44	70.38
Total Antioxidant	+29.57	-
Total Phenol	-	+8.95
Total Flavonoid	-	+33.62
FRAP	+31.95	-
DPPH	-	+23.61
ABTS	+16.15	-
Cell Viability	-	-13.22
NO Production	-	+11.56

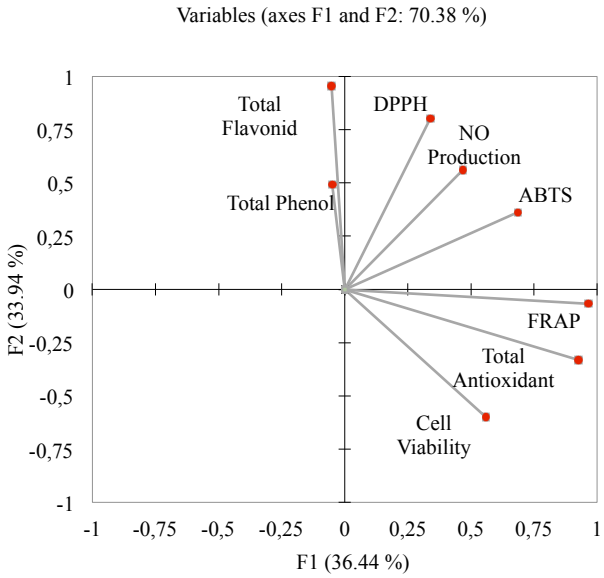

Figure 5. Plots of the scores for antioxidant and anti-inflammatory activities content.

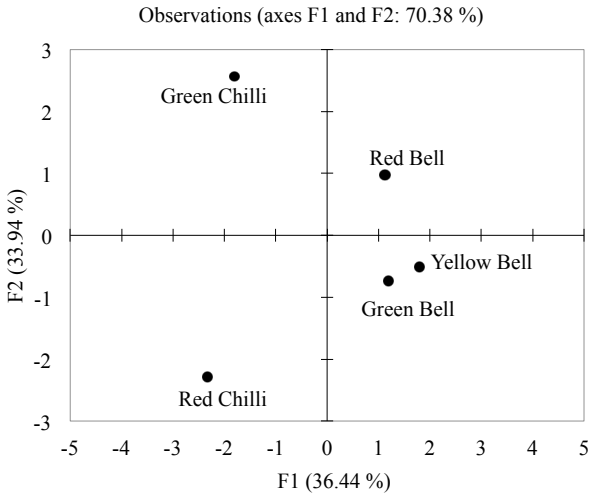

Figure 6. Plots of the x- loadings antioxidant and anti-inflammatory activities content.

4. CONCLUSION

The results of this study offer an experimental basis for the event of recent ways to provide knowledge of many pharmacological and biochemical properties. Although these results warrant further in-vivo studies, the presented in-vitro data suggest the potential of pepper to attenuate inflammation and oxidative stresses.

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