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STUDY ON FUNCTIONAL PROPERTIES OF SELECTED CHILLI VARIETIES GROWN IN KATHMANDU, NEPAL

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ABSTRACT

The present work was undertaken to study the functional properties (oleoresin, pungency, antioxidant activity, phenolic content, ascorbic acid content and natural pigments) of six chilli varieties found in Kathmandu, Nepal. The yield of oleoresin was found to be higher in ethyl acetate than acetone. *Habanero* showed the highest pungency (420,000 SHU) while *Bell pepper* showed lowest pungency (0 SHU). The antioxidant activity was higher in *Habanero* (IC_{50} = 206.43 µg/mL) due to high phenolic content 128.6 mg of GA/g and low in Indian pepper (IC_{50} = 578.16 µg/mL) due to lower phenolic content 74.92 mg of GA/g. The ascorbic acid contents were ranged from 38.59±4.53 to 107.52±18.65 mg/100g and maximum was observed in Pimento. Among six varieties, chlorophyll and carotenoid contents were found to be maximum in *Jire*.

Keywords: Chilli, pungency, antioxidant activities, ascorbic acid, natural pigments

INTRODUCTION

Chillies are cultivated species from plain region to mid hills of Nepal. It is mostly accepted as spice crop and it occupied fourth position as a spice crop with a productivity of 3.45 t/ha. Chilli (*Capsicum spp.*) fruits are available in the market throughout the year since chilies are grown in all seasons in all parts of the country **(Dahal *et al.*, 2006)**. FAO estimates 20,000 tonnes dried chilli and pepper was produced in Nepal in 2012 **(FAOSTAT, 2013)**. Chilli is an indispensable adjunct and a popular condiment in the world of food. Chilli is a well-known commercial crop used both as a condiment or culinary supplement and also as a vegetable **(Pickersgill, 1997)**. Besides adding flavour, it also adds to the nutraceutical value to the diet **(Ruanma *et al.*, 2010)**. There is at present increasing interest in spices and aromatic herbs because of their strong antioxidant properties, which are due to many substances, including some vitamins, flavonoids, terpenoids, carotenoids, phytoestrogens, minerals, etc. **(Suhaj, 2006).** The main functional properties of chilli are pungency, antioxidant activity, vitamin C and natural pigments **(Staryth and Nosova, 1982; Garcia *et al.*, 1998; Jagadeesh, 2000)**. Oleoresins consist of essential oil, natural colorants, waxes, alkaloids and pharmaceutical ingredients **(Wesolowska *et al.*, 2011)**. Oleoresins can be extracted using range of solvents (**Mini *et al.*, 2008)**. Pungency is due to the presence of capsaicin content **(Parthasarathy *et al.*, 2008).** Color of ripe pepper fruits originate from carotenoids, and two red carotenoids, capsanthin and capsorubin, naturally found only in Capsicum **(Govindarajan, 1986; Levy *et al.*, 1995; Deli *et al.*, 2001; Pino *et al.*, 2006)**. Thus, the present work aims to study the some of the functional properties of selected chilli varieties found in Kathmandu, Nepal.

MATERIAL AND METHODS

Six chilli varieties namely *Pimento, Jire, Bell Pepper, Indian Pepper, Teja and Habanero* were purchased from the local market of Kathmandu, Nepal. The foreign, earthy matter and residual materials were removed from the fruits. It was then mechanically reduced to coarse powder (70-80 mesh size) using electric grinder manufactured (Black and Decker, India) and the powder was kept in a sealed glass jars under refrigerated condition 4±2 ºC until use.

Analytical procedure

Determination of pungency (SHU)

Pungency level of chilli was measured by Scoville heat units (SHU). Ten gram grounded chilli sample (70-80 mesh size) was weighed and the oleoresin was extracted by using ethyl acetate and acetone as solvent followed by removal of the solvent by evaporation **(Ranganna, 2009)**. One gram of the oleoresin, extracted from chilli, was weighed and mixed with 50 mL of ethyl alcohol (minimum assay 95%). The mixture was allowed to stand for 24 h, with occasional shaking. Serial dilutions of the clear supernatant were done with a 5% solution of sugar. Diluted solution (5 mL) was tasted to detect the presence or absence of distinct pungency in the throat or mouth **(Parthasarathy *et al.*, 2008)**. Sensory evaluation was performed using a panel of ten taste panelists and then the degree of hot sensation was noted.

Antioxidant activity determination

Preparation of extracts

The extracts were prepared by cold maceration method in which about 50 g of dried, ground powder was extracted into 500 mL of methanol, a method more often used for extraction of plant materials; extracts were subjected to shaking in water bath shaker (Sonar, India) at room temperature for 24 h. The extracts were filtered using Whattman filter paper no 1. The extracts were then kept in reagent bottles covered with aluminum foil and stored at 4±2 ºC until used **(Brand-Williams *et al.*, 1995)**.

DPPH method for antioxidant activity determination

The ability of the plant extract to scavenge DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radicals was assessed by the standard method (**Brand-Williams *et al.* 1995)** with slight modification. Different extract concentrations e.g., 25, 50, 100, 200, 400, 800, 1600 and 3200 µg/mL were prepared from stock solution (5000 µg/mL). Antioxidant solution in methanol (0.1 mL) was added to 3.9 mL of a 6 $\times 10^{-5}$ mol/L methanol (assay GC 99.0%) DPPH solution. After 30 min incubation in dark at room temperature (27±2^{0}C), the reduction of the DPPH free radical was measured by reading the absorbance at 517 nm. DPPH is a purple-colored stable free radical that when reduced becomes yellow. Gallic acid (minimum assay 99.5%) was used as positive control. The corresponding percentage of inhibition was then calculated by using the formula:

$$\% \text{ Inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

Where, A_0 is the absorbance of the control (Solution of Methanol only) and A_1 is the absorbance of the extract.

The fifty percent free radical inhibition (IC_{50}) of extract was plotted against respective concentrations used and from the graph IC_{50} was calculated by using linear regression line.

Total phenolic content (TPC) determination

Total phenolic compound was determined by Folin-Ciocalteau reagent using the method of **Singleton and Rossi (1965)** with slight modification. 200 µL of

diluted sample was added to 1 mL of 1:10 diluted Folin-Ciocalteau reagent. After 4 minutes, 800 µL of saturated sodium carbonated solution (assay 99%) (75 g/L) was added. After 2 h of incubation at room temperature, the absorbance at 765 nm was measured by spectrophotometer in triplicate. Gallic acid (0-500 mg/L) was used for calibration of standard curve. The results were expressed as mg of gallic acid equivalent (mg GAE)/g dry weight of chillies.

Ascorbic acid determination

The ascorbic acid content was determined by 2, 6-dichlorophenol indophenol visual titration method according to **Ranganna (2009)**.

Natural pigment determination

The chlorophyll content was analyzed as per the method suggested by **Yoshida *et al.* (1972)**. The total carotenoid content of different varieties was estimated according to **Ranganna (2009)**.

Data analysis

The observed values were reported as the mean of triplicate samples and their standard deviation (SD). Significant differences for multiple comparisons were determined using Microsoft Excel 2007, (©2006, Microsoft corporation, USA). Two way analysis of variance (ANOVA, no blocking) and Least significant difference (LSD) test was used to assess the significant differences with the Genstat Discovery Edition 4(©2011, VSN International Ltd., UK). Differences at $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Oleoresin extraction of chilli

Oleoresin content of *Jire* was recorded highest in acetone and ethyl acetate extracts while it was lowest in *Pimento*. The percent oleoresin of chilli was higher in ethyl acetate than acetone. The percentage oleoresin of chilli varieties ranged from 6.14 to 18.45% in ethyl acetate and 4.33 to 13.22% in acetone.
The yield of oleoresin using both of the solvents significantly differed at 0.05 level of significance (Figure 1). For oleoresin extracts, the varieties *Jire*, *Teja* and *Habanero* were found to be more suitable due to their higher oleoresin level (>15%). Similar findings can be associated with the use of solvents for extraction (**Parthasarathy *et al.*, 2008; Mini *et al.*, 1998**). **Wesolowska *et al.* (2011)** had reported oleoresin comprises mixture of essential oil, color and pungency. Maximum efficiency was noted with ethyl acetate for oleoresin extraction while comparing different solvents (**Mini *et al.*, 1998)**.

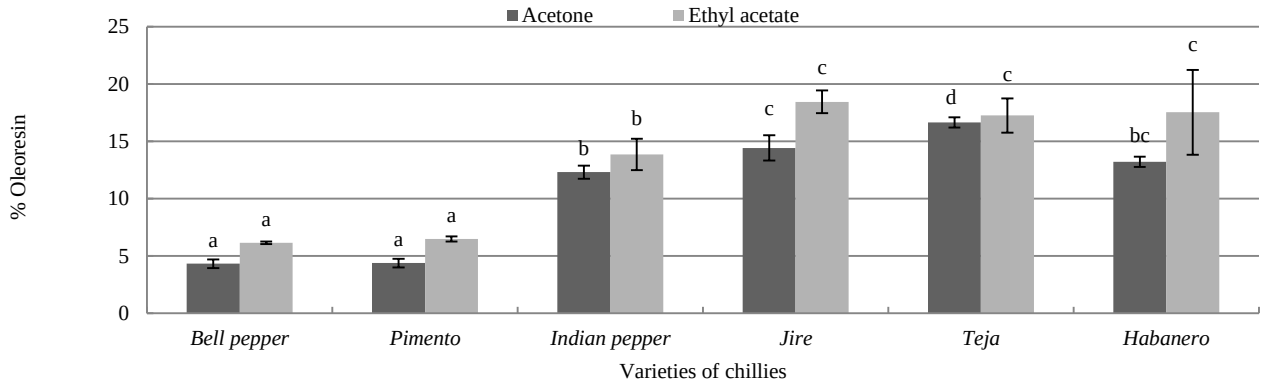

Figure 1 Comparison of oleoresin yield using different solvent. Superscript with different alphabets in the bar diagram differ significantly ($p<0.05$).

Pungency level

The recorded pungency level of *Bell pepper*, *Pimento*, *Indian pepper*, *Jire*, *Teja* and *Habanero* were 0, 0, 24,000, 30,000, 360,000 and 420,000 SHU respectively as shown figure 2. *Habanero* showed the highest SHU level followed by *Teja* due to high amount of capsaicin content, whereas *Bell pepper* and *Pimento* showed the lowest SHU levels due to low capsaicin content. **Parthasarathy *et al.* (2008)** also reported pungency level of *Indian pepper*, *Jire*, *Teja and Habanero* were 20,000-25,000, 12,000-30,000, 70,000-370,000 and 350,000-577,000 SHU respectively. Soil types, climate, and region can also play a role in this variation in heat, and the difference can often be quite large; sometimes by a factor of 10 or more **(Staryth and Nosova, 1982)**.

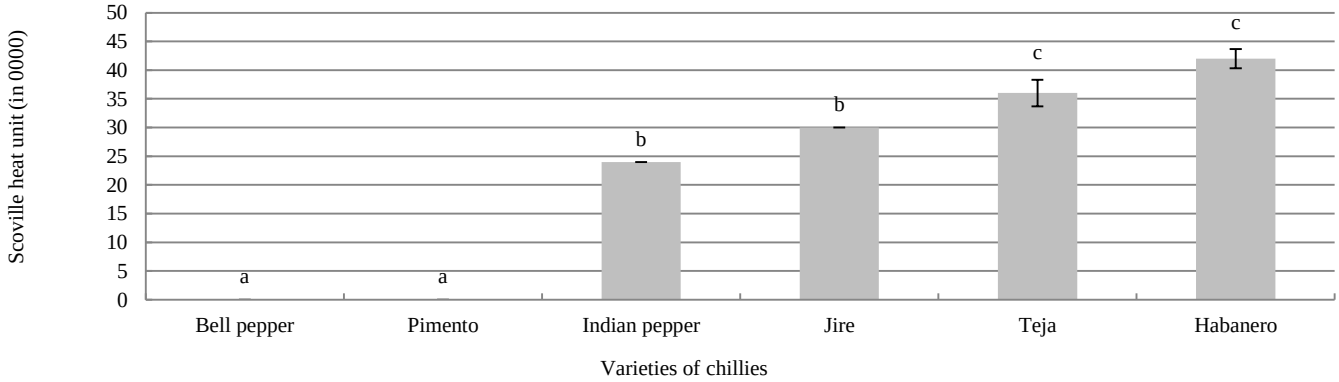

Figure 2 Pungency level of chilli. Superscripts with different alphabets in the bar diagram differ significantly

DPPH scavenging activity and phenolic content

Methanolic extracts of chilli varieties had significant radical scavenging effect on DPPH radical. The reduction in DPPH radical concentration together with the increase of chilli extracts concentration was observed as first order kinetics ($y = 0.016x + 37.75$, r^2 = 0.933) and obtained values are placed in Table1. *Habanero* showed the highest antioxidant activity (IC_{50}=207.22 µg/mL) and *Indian pepper* showed the lowest antioxidant activity (IC_{50}=582.5 µg/mL). The IC_{50} value for standard GA ($y = 0.001x + 0.151, r^2$ = 0.98) was found to be 765.62 µg/mL. An earlier study also reported that chilli has a strong antioxidant activity **(Ruanma *et al.*, 2010)**. Hence, in comparison, methanolic extract of *Habanero* appeared to be more powerful radical scavenger. The values obtained for the concentration of total phenols (mg of GA/g of extract) are presented in Table 1. Different phenolic levels reported in the literature **(Suhaj, 2006)** could partially be associated with the method of extraction. In fact, preliminary work to the present study revealed that extraction yield of phenolics using ethanol was 2 to 3 fold lower than that with methanol.

Table 1 IC_{50} and total phenolic content of chilli

Sample	IC_{50} value (µg/mL)	Phenolic content (mg of GA/g)
Bell pepper	*264.43±1.56*[a]	*66.18±2.31*[a]
Pimento	*295.99±1.03*[b]	*52.89±1.23*[b]
Indian pepper	*578.16±6.13*[c]	*74.92±1.33*[c]
Jire	*379.31±0.96*[d]	*85.91±1.14*[d]
Teja	367.39±2.10[e]	95.76±0.79[e]
Habanero	206.43±1.11[f]	128.58±1.57[f]

The values are means of triplicate determination with standard deviation. Superscript with different alphabets in the bar diagram differ significantly ($p<0.05$).

Ascorbic acid

The ascorbic acid content was significantly different at 0.05% level of significance (Table 2). The ascorbic acid content of chilli varieties ranged from 38.59 to 107.52 mg/100 g. **Garcia *et al.* (1998)** observed an increase in ascorbic acid content after green mature stage and peaked at ripe fruit with 75% of moisture. **Dahal *et al.* (2006)** also observed variations in ascorbic acid content with 32.86 mg/100g to 173.6 mg/100g. Ascorbic acid content differed between varieties. Similar observations were made by other investigators (**Basavaraja, 1997; Shashidhar, 2000; Jagadeesh, 2000)**. The ascorbic acid content of dry varieties ranged between 53 to 74 mg/100 g which was on par with the results obtained and reported ascorbic acid content range of 38 to 86 mg/100 g in whole chilli fruits.

Natural pigments

The chlorophyll content of chilli varieties are presented in table 2. The chlorophyll content of chilli varieties was ranging from 0.13 to 2.10 mg/100 g fruits. The maximum amount was observed in *Jire* (2.10 mg/100 g) and minimum in *Bell pepper* (0.13 mg/100 g). The total carotenoid content of chilli varieties ranged between 0.65 to 2.33 mg/100 g. Jire had the highest carotenoids at 2.33 mg/100 g followed by *Habanero* at 2.12 mg/100 g, whereas the *Bell pepper* had the lowest (0.65 mg/100 g). From the table 2, a significant difference in chlorophyll content was observed between chilli varieties. Present study showed that the total chlorophyll content of *Jire* is the highest among others at 2.09 mg/100 g. From the table 2, a significant difference in carotenoid content was also observed between chilli varieties. Chilli *Jire* and *Habanero* did not significantly differ at 0.05 level. The total carotenoids content of chilli varied from 1.01 to 2.33 mg/100 g and maximum was noticed in *Jire.* These results are at par with the data produced by **Deli *et al*. (2001)** in red paprika that is 1.3 mg/100 g of dry weight. It is obvious that carotenoid pigments co-exist with chlorophyll. The carotenoid content of chilli cultivars varied from 0.81 to 3.82 mg/100 g, these results are on par with **Staryth and Nosova (1982)** who observed significant variations in carotene values, from 1.2 to 3.5 mg/100 g fruits. Similarly, **Levy *et al*. (1995)** also observed wide variation in carotenoid content among cultivars and higher amounts noticed in *Capsicum annuum* species.

Table 2 Ascorbic acid, chlorophyll and carotenoid content in mg/100g

Sample	Ascorbic acid	Total chlorophyll	Total carotenoids
Bell pepper	99.31±28.22[a]	0.11±0.02[a]	0.65±0.01[a]
Pimento	107.52±18.65[a]	0.37±0.01[b]	1.13±0.02[b]
Indian pepper	38.59±4.53[b]	0.25±0.01[c]	0.70±0.03[c]
Jire	39.00±7.95[b]	2.09±0.01[d]	2.33±0.01[d]
Teja	40.91±7.05[b]	1.12±0.03[e]	1.05±0.03[bc]
Habanero	106.11±22.23[a]	0.52±0.01[f]	2.12±0.03[d]

Each value is expressed as a mean with standard deviation. Superscript with different alphabets in the table differ significantly ($p<0.05$).

CONCLUSION

Habanero and *Jire* have the highest level of SHU, therefore, better for pungency level. Chilli varieties had a significant antioxidant principles as % DPPH scavenging activity and ascorbic acid. *Habanero* had maximum antioxidants as %DPPH scavenging activity and mg % Ascorbic acid content was observed maximum in *Pimento*. The yield of oleoresin of chilli was maximum in ethyl acetate than that of acetone. Chilli varieties found in Kathmandu, Nepal had significant amount of carotenoids compared to cholorophyll as natural pigment. In conclusion, the analysed six varieties show good functional properties.

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