INTRODUCTION

Chillies are cultivated species from plain region to mid hills of Nepal. It is mostly accepted as a spice crop and it occupied fourth position as a spice crop with a productivity of 3.45 t/ha. Chili (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). Chili is an indispensable adjunct and a popular condiment in the world of food. Chili 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 (Ranuma 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 (Parthasarathy, 1995). Oleoresin 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 °C 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 Whatman filter paper no 1. The extracts were then kept in reagent bottles covered with aluminum foil and stored at 4±2 °C 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 × 10−3 mol/L methanol (assay GC 99.0%) DPPH solution. After 30 min incubation in dark at room temperature (27±2°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:

\[ \% \text{ Inhibition} = \left( \frac{A_t - A_s}{A_t} \right) \times 100 \]

Where, \( A_t \) is the absorbance of the control (Solution of Methanol only) and \( A_s \) is the absorbance of the extract.

The fifty percent free radical inhibition (IC50) of extract was plotted against respective concentrations used and from the graph IC50 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 28.45% 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, 36,000, 36,000 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 , r² = 0.933) and obtained values are placed in Table1. Habanero showed the highest antioxidant activity (IC₅₀=207.22 µg/mL) and Indian pepper showed the lowest antioxidant activity (IC₅₀=582.5 µg/mL). The IC₅₀ value for standard GA (y = 0.001x + 0.151, r² = 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.

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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. García 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).

Natural pigments

The chlorophyll content of chilli varieties are presented in table 2. The chlorophyll content of chilli varieties ranged between 53 to 74 mg/100 g which was on par with results obtained and reported ascorbic acid content range of 38 to 86 mg/100 g in whole chilli fruits.

Table 1 IC50 and total phenolic content of chilli

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC50 value (µg/mL)</th>
<th>Phenolic content (mg of GAE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bell pepper</td>
<td>264.43±1.56a</td>
<td>66.18±2.31a</td>
</tr>
<tr>
<td>Pimento</td>
<td>295.99±1.03c</td>
<td>52.89±1.23c</td>
</tr>
<tr>
<td>Indian pepper</td>
<td>578.16±13b</td>
<td>74.92±1.33b</td>
</tr>
<tr>
<td>Jire</td>
<td>379.31±90d</td>
<td>85.91±1.14d</td>
</tr>
<tr>
<td>Teja</td>
<td>367.39±2.10d</td>
<td>95.76±0.79d</td>
</tr>
<tr>
<td>Habanero</td>
<td>206.43±1.11e</td>
<td>128.58±4.57e</td>
</tr>
</tbody>
</table>

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

REFERENCES


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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ascorbic acid (mg/100g)</th>
<th>Total chlorophyl (mg/100g)</th>
<th>Total carotenoids (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bell pepper</td>
<td>99.31±28.22d</td>
<td>0.11±0.02d</td>
<td>0.65±0.01d</td>
</tr>
<tr>
<td>Pimento</td>
<td>107.52±18.65c</td>
<td>0.37±0.01d</td>
<td>1.13±0.02d</td>
</tr>
<tr>
<td>Indian pepper</td>
<td>38.59±4.53d</td>
<td>0.25±0.01d</td>
<td>0.70±0.03d</td>
</tr>
<tr>
<td>Jire</td>
<td>39.00±7.95e</td>
<td>2.09±0.01d</td>
<td>2.33±0.01d</td>
</tr>
<tr>
<td>Teja</td>
<td>40.91±7.05e</td>
<td>1.12±0.03d</td>
<td>1.05±0.03d</td>
</tr>
<tr>
<td>Habanero</td>
<td>106.11±22.23e</td>
<td>0.52±0.01d</td>
<td>2.12±0.03d</td>
</tr>
</tbody>
</table>

Each value is expressed as a mean with standard deviation. Superscript 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 antioxidas as %DPPH scavenging activity and mg % Ascorbic acid content was observed maximum in Pimento. The yield of oleoresin was maximum in ethyl acetate than that of other solvents. Chilli varieties found in Kathmandu, Nepal had significant amount of carotenoids compared to chlorophyll as natural pigment. In conclusion, the analysed six varieties show good functional properties.

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