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A Review of Anti oxidant Effects of the Ethanol Extract of the Medicinal Plant Carla (*Momordica charantia*) Cultivated in Northern Region of Iran (Mazandaran-Simorgh city)

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ABSTRACT: Carla (*Momordica charantia*) is one of medicinal plants found in tropic regions which has anti diabetic effect, anti blood fat effect and so on. Since one of consumable parts of this plant, i.e. its fruit is useful against diabetes in the form of Capsules or herbal tea; we collected it from the north of Iran (Mazandaran province-Simorgh city) for this study and after identification used it for producing ethanol extract. Extraction process was carried out by the soaking method. The resulting extracts were solvent-removed and degreased. Studying the anti oxidant effects of the ethanol extract was measured using DPPH method and also phenol and flavonoid content and regenerative strength of iron. Results indicated that the ethanol extract of the plant with IC_{50} = 250 mg/ml has anti oxidant effects, and also phenol and flavonoid were 28.6±0.2 mg/ml and 19.93±0.1 mg/ml respectively. As a result, the ethanol extract of Carla plant causes extraction of high levels of total phenol that inhibit free radicals of DPPH.

Key words: anti oxidant, extract, Carla, diabetes

INTRODUCTION

Carla is a local plant of tropic regions of the world, which is known in various countries as Carla, Balsma, and Bitter melon and its wild and domesticated species are scattered in tropic regions. This plant is mostly local in India and southern china and is also found in tropic and semi tropic regions (Leatherdale *et al.*, 1981). It is considered as a local fruit of several countries in south and east of Asia such as Sri Lanka, Vietnam, Thailand, Malaysia, Philippines, Pakistan and South China and it is also cultivated in some of tropic regions of America. Lately it was cultivated empirically in Iran and a convincing result was obtained from cultivating the Indian species of Carla (Welihinda *et al.*, 1982).

The leaves and stems of Carla are rich sources of calcium, Iron, phosphor and vitamin c and vitamin a. local people of Amazon cultivate Carla in their gardens for food and medicine. They add Carla to their soups and beans as a flavor in order to have spicy and salty flavors. There is an ancient history of people's local knowledge and medicinal applications of this plant in the Amazon region. A type of tea that is produced from its leaves is used for curing diabetes (Sarkar *et al.*, 1996). In the traditional herbal medicine, Carla is believed to stimulate the digestive system and increase the appetite based on its soar flavor like other medicinal plants. The role of free radicals in causing a variety of diseases has already been proved (Kumaran and Karunakaran, 2006). Several biochemical reactions in the body produce reactive oxygen species that are capable of causing damage to biomolecules. This harmful influence of free radicals can be blocked by anti oxidant substances. These compounds cause free radicals to be trapped and also cause detoxification. Foods rich in anti oxidant play a significant role in preventing cardiovascular diseases, cancer and degenerative diseases (Kris-Ethertonm *et al.*, 2002; Di matteo and Esposite, 2003).

Trapping the DPPH (Diphenyl-2-picryl-hydrazyl) is based on hydrogen's capabilities (Chung *et al.*, 2006). This method is used in order to evaluate the activity of free radicals. We can refer to lack of dependency on sample polarity as one of its advantages (Kartal *et al.*, 2007). In the method of regenerative strength, the existing substances in the sample are measured by regenerating iron III to iron II with the capacity of losing electron (Chung *et al.*, 2006). Since still there is no report about the anti oxidant effect of this plant, we decided to along with measuring the flavonoids and total phenol content, study the anti oxidant feature of methanol extract of this organism using in vitro methods, and provide a better economic utilizing of this plant. In this paper we study the antioxidant content and, phenol and flavonoid content of ethanol extract produced from Carla.

MATERIALS AND METHODS

A. Extraction of ethanol

In order to produce this extract, we first take 60 grams of dried Carla plant cultivated in the northern region of Iran (Mazandaran province- Simorgh city) and pour it into decantation, then add an amount of ethanol equivalent to twofold of dried material. We repeat this three times by ethanol and then the pure extract is maintained using rotary or distillation method.

B. Measuring total phenol and Flavonoid content

Content of phenol compounds was measured using ciocalteau method (Ordon ez *et al.*, 2006). The total phenol content was measured using Folin-ciocalteau reactant. The extract was mixed with 0.2 normal Foiln-ciocalteu and after being alkalinized, it absorption level was recorded 2 hours later at 760 nm wavelength against blank using Model EZ201 Perkin Elmer spectrophotometer made in America. The total phenol content was reported based on a value equivalent to milligram of Gallic acid in each gram of extract. Aluminum chloride identifier was used in order to measure the level of Flavonoid. A 10% $AlC1_3$ solution, methanol, one molar stat potassium solution and distilled water were added to the extract. The absorption level of this mixture was read half an hour later at 420 nm wavelength against blank. The level of Flavonoid was reported based on a value equivalent to milligram quercetin in each gram of extract (Ordon ez *et al.*, 2006). The experiments were repeated three times and their average was reported.

C. Evaluating the trapping capability of DPPH radicals

Stabilized DPPH radicals were used for this experiment. Different concentrations of the extract were mixed with an equal volume form DPPH methanol solution (100 micro molars) and after stirring, were incubated for 10 minutes in a dark room. Then the level of absorption was read using a UV spectrophotometer at 517 nm with methanol blank. Ascorbic acid was used as standard and IC_{50} was identified for extract as the required concentration of extract in order to clean 50 percent of radicals. Finally the DPPH radical trapping percentage can be estimated using bellow formula:

$$\left(\frac{A_B - A_S}{A_B}\right) \times 100$$

A_B = blank absorption, A_S = sample or standard absorption

D. Identifying the regenerative strength

We evaluated the regenerative strength of the extracts using Yen and Chen's method (Yen And Chen., 1995). Different concentrations were prepared from each extract and they were mixed with 2.5 ml of 0.2 m phosphate buffer with 6.5-pH and 2.5 ml of 1 percent free Siatyd potassium solution [$K_3Fe(CN_6)$]. The resulting solution was kept for 20 minutes under 50°C temperature and then, 2.5 ml of tri coloristic acid solution was added to the samples in order to stop the reaction. Then samples were placed in a centrifuge at 3000 rpm for 10 minutes. After this step, 2.5 ml from upper part of the solution was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1 percent free chloride ($FeCl_3$) solution was added to this mixture. Then the absorption level of solution was read at a wavelength of 700 nm. In this experiment, ascorbic acid was used as the standard. The experiment was repeated three times for each sample.

E. Statistical analysis

All of measurements were repeated 3 times and all the information was reported as Mean±SD. Unidirectional analysis of variance (ANOVA) was used for comparing the averages. The results were considered with a significant probability of $p<0.05$. The IC_{50} values were obtained on a linear regression between inhibiting percentage and corresponding concentrations.

RESULTS

A. Phenol and Flavonoid content

The total phenol content was calculated by using Folin Ciocalteau method as oaki valan mg in any gram of extract based on the linear equation of standard curve(Y = 0.0063x, r2 = 0.987). The total phenol content of ethanol extract was 0.2±28.6 milligram of Gallic acid in each gram of extract's powder. The flavonoid content on the basis of standard curve linear equation (y=0.0067x+0.0132, r2=0.999) for this extract was obtained as 19.93±0.1 oaki valan milligram of quercetin in any gram of extract's powder.

B. Activity of trapping the DPPH radicals

In this study we observed that the trapping activity of radicals in all extracts increased with increasing concentrations (Fig. 1.). The inhibiting concentration of 50% (IC_{50}) resulted for micro gram in milliliter of the ethanol extract of this plant. IC_{50} was obtained for ascorbic acid of microgram in milliliter.

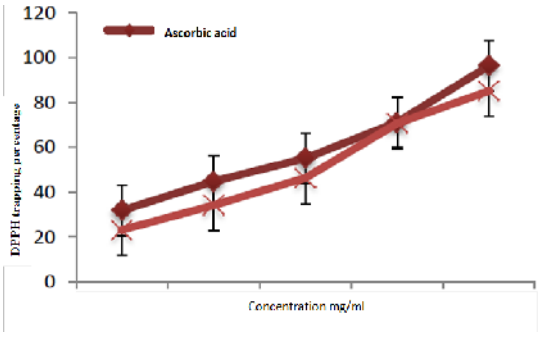

Fig. 1. Comparing the trapping percentage of DPPH using ethanol extract of the Carla plant and ascorbic acid.

C. Regenerative strength of extract

Fig. 2 demonstrates the concentration -response curve of the ethanol extract. In this study we observed that the regenerative strength of extracts increase with concentration rising.

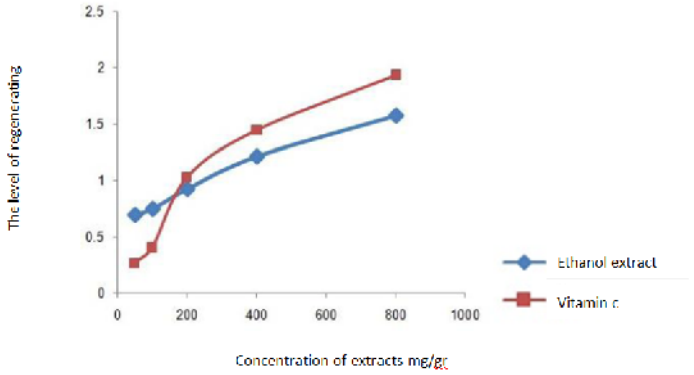

Fig. 2. comparing the regenerative strength of ethanol extract of the Carla plant and vitamin C.

DISCUSSION

Carla is a local plant that grows in tropic regions of the world and its wild and domestic species are scattered in tropic regions. This plant is mostly local in India and southern china and is also found in tropic and semi tropic regions. It is considered as a local fruit of several countries in south and east of Asia such as Sri Lanka, Vietnam, Thailand, Malaysia, Philippines, Pakistan and South China and it is also cultivated in some of tropic regions of America. Lately it was cultivated empirically in Iran and a convincing result was obtained from cultivating the Indian species of Carla (Welihinda *et al.*, 1982). The total phenol content based on standard curve linear equation for ethanol extract was obtained 28.6±0.2 oaki valan milligram of Gallic acid in gram of extract. The flavonoid content also for the same set on the basis of standard curve linear equation was obtained 19.93±0.1 oaki valan milligram of quercetin in gram of extract. Phenols and poly phenol compounds such as flavonoids are widely found in food product and have shown to have considerable anti oxidant activity (Van Acker *et al.*,1996). Studies indicate that increase in flavonoid level in diets can cause reduction in some diseases in humans (Hertog *et al.*, 1993). The level of total phenol and flavonoid in this study can justify the anti oxidant activity presented in the extracts produced from this plant. The trapping method of stabilized DPPH radicals is widely used for evaluating the capability of trapping radicals in various samples (Lee *et al.*, 2003). Measuring the inhibiting level of DPPH free radicals is considered as a valid, Precise, and economical method with high repeatability which is utilized in studying anti oxidant activity of herbal extracts in laboratory conditions. The results of DPPH test indicated that capability of the extract in inhibiting free radicals depends on its concentration and increases with increasing concentration of anti radical activity. In higher concentrations of phenol compounds, the probability of hydrogen transfer to free radical and consequently higher inhibiting strength is increased due to increase in number of hydroxyl groups existing in reaction environment. Measurement of regenerative strength in extracts is due to regenerating iron 2 to iron 3 with electron transmission. The complex level of iron is measurable by measuring the level of blue color formation at 700 nm. The increase of absorption in this wavelength is suggestive of an increase in regenerative capability. In extracts, the absorption level increases significantly with concentration rising in respect of regenerating strength. Additionally, we observe in comparison with the sample, i.e. vitamin c that although in lower concentrations the inhibiting strength is less, but in higher concentrations the inhibiting strength is almost close to that of sample. This result suggests that for reaching regenerative strength, the required concentration from extract must be equal to concentration of vitamin c. results of this study indicate that phenol compounds produced from this plant can be used in medicine industry as well.

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