IJNES
International Journal of Natural and Engineering Sciences 4 (1): 69-72, 2010
ISSN: 1307-1149, E-ISSN: 2146-0086, www.nobel.gen.tr

Free Radical Scavenging Activities of *Lophocereus schottii* (Engelmann)

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Received: November 01, 2009
Accepted: Januraary 29, 2010

Abstract

The antioxidant activity of methanol extract of Lophocereus schottii was determined with three different tests: 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) scavenging; trolox equivalent antioxidant capacity (TEAC); and ferric reducing antioxidant power (FRAP). The extract showed significant activities in all antioxidant assays, in DPPH radical scavenging assay the EC_{50} value was found to be 132.6 µg/ml while catequin had the EC_{50} value 111.2 µg/ml. Phenolic and flavonoid contents of the extract also determined and the values were 73 mg (gallic acid equivalent/1 g extract) and 5 mg (catechin equivalent/1 g extract). This finding indicated that crude extract of L. schottii was a starting material for the isolation of compound(s) with effective activities as radical's scavengers.

Keywords: Antioxidant activity, phenolic compounds, Lophocereus schottii,

INTRODUCTION

A free radical is a compound with one or more unpaired electrons in its outer orbital [1]. Such unpaired electrons make these species very unstable and
therefore quite reactive with other molecules due to the presence of unpaired electron [2] and they try to pair their electron(s) and generate a more stable compound. In living systems, free radicals are generated as part of the body's normal metabolic process, but a bad lifestyle and environmental polluting agents, can result in increased radical activity and damage certain biomolecules [3].

Plant phenolics provide protection against the harmful effects of the oxidative stress, which has been related to the risk of coronary heart disease, cardiovascular disease, atherosclerosis, inflammation [4-6], certain types of cancer [7] nd other neurodegenerative diseases [8].

The Cactaceae is an important phytogeographic element of the American deserts and the second largest plant family restricted to the New World [9]. In Mexico, several species belonging to this plant family have been used from pre-hispanic times as food and medicinal plants. *Lophocereus schottii* is a cactus that grows mainly in the south of USA (popular called senita cactus) and north of México (commonly called músaro), this cactus is used as a folk medicine for the treatment of diabetes and cancers [10- 11]. Phytochemical investigations have led to the isolation of alkaloids pilocereine and lophocine, and terpenoids like lupeol, lophenol and schottenol [12-15].

A review of literature did not reveal any information on the antioxidant study of this cactus. Therefore the aim of the present study was to investigate radical scavenging activities of *L. schottii* by DPPH, TEAC and FRAP assays.

MATERIALS and METHODS

Cactus sample and preparation of the extract

The aerial part of *Lophocereus shottii* used in this study were collected in July 2003, in Sonora, Mexico, and was authenticated by Dr Marcela González Álvarez. A voucher specimen was deposited in the herbarium of the Facultad de Ciencias Biológicas de la Universidad Autónoma de Nuevo León. (voucher specimen number 024185). The aerial parts of the cactus were dried at room temperature and 100 g was extracted with 500 ml of MeOH (3 x 24 h) by maceration. After filtration and concentration under reduced pressure, 16 g of the dark-green extract was obtained (w/w yield 1.6%).

Total phenolic and flavonoid content

Total phenolic content was determined using the Folin-Ciocalteu reagent as described by Singleton and

Rossi [16] with some modifications. Samples (100 µl) were placed into test tubes, 250 µl of Folin-Ciocalteu's (1N) reagent, 1250 µl of sodium carbonate (20 %) and 400 µl of distilled water were added. The tubes were mixed and incubated for 2 h and the change in absorbance was measured at 760 nm. Gallic acid was used as a standard. The total phenolic content was expressed as mg of gallic acid eq./g of dry extract.

The flavonoid content was determined by the aluminum chloride colorimetric method according to Chang *et al* [17] (+) Catechin was used as a reference for the calibration curve. The absorbance of the reaction mixture was measured at 415 nm. Results were expressed as mg (+) catechin eq./g extract.

TEAC assay

The Trolox equivalent antioxidant capacity (TEAC) of the extract was determined by the ABTS [2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] cation radical discoloration assay [18] and the values are reported as mM Trolox. The method is based on the consumption of the preformed ABTS•+ in the presence of potassium persulfate followed at the maximum absorption of 734 nm. Addition of antioxidants to ABTS•+ reduces it to ABTS. The assay was performed on 96 well microplates, the absorbance of ABTS•+ was adjusted to 0.70 ± 0.02. The decrease of the absorption was measured after 6 min. The results are presented as mean ± SD. Each measurement was performed at least in triplicate.

FRAP assay

The ferric reducing-antioxidant power (FRAP) assay uses antioxidants as reductants in a redox-linked colorimetric method. The antioxidant activity was measured by the sample ability to reduce the Fe^{3+}/ ferricyanide complex by forming ferrous products in a 96 well microplate reader. Absorbance was measured at 595 nm exactly 8 min after mixing the FRAP solution and the sample. Twenty five µl of MeOH were used as a blank. The FRAP values are expressed as mM of $FeSO_4$ per milligram of dried plant [19].

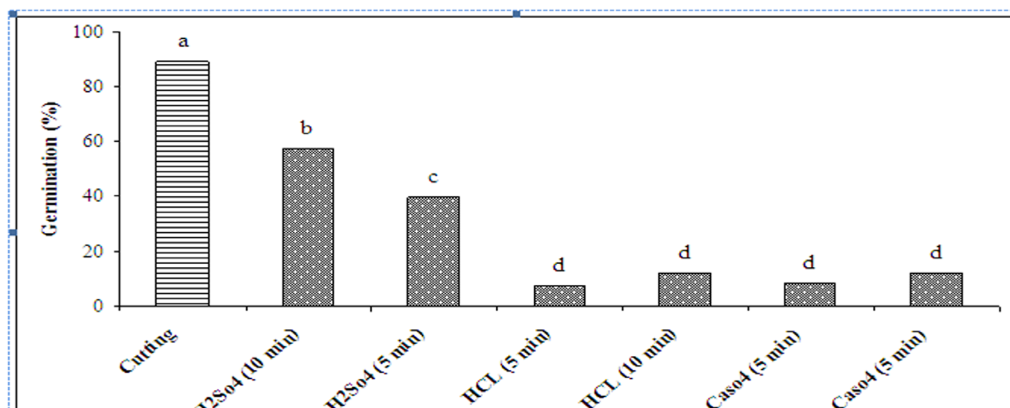
DPPH radical - scavenging activity

Serial dilutions of the test samples dissolved on MeOH were mixed with DPPH• (1 mM) solution in 96-well microplates. MeOH was used as a negative control and (+) catechin was used as positive control. The change in absorbance at 517 nm was measured. Mean values were obtained from triplicate experiments. Inhibition percent was calculated using the equation: % inhibition = [(A0 –A1) / A0] x 100 where A0 is the absorbance of the control and A1 is the absorbance of the samples [20]. The radical scavenging activities were expressed as the median effective concentration (EC_{50}). The EC_{50} was calculated from the log-dose inhibition curve obtained by a nonlinear regression algorithm.

RESULTS and DISCUSSION

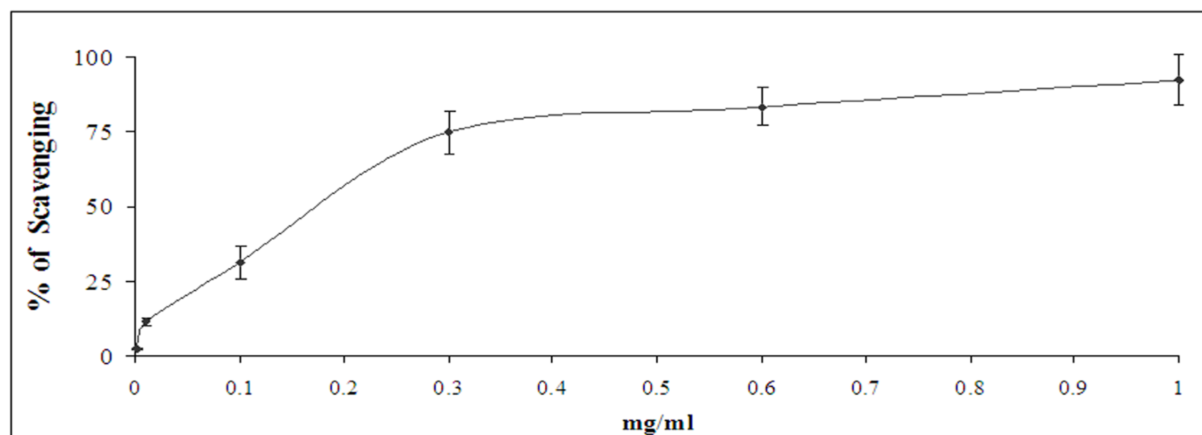
The results of the phytochemical analyses revealed that the methanolic extract of *L. schottii* has total phenol and flavonoid contents of 73± 12 mg gallic acid eq/g extract and 5 ± 0.56 mg catechin eq/g extract, respectively. In this present study the antioxidant activity of the methanol extract of *L. schottii* was investigated by using DPPH scavenging assay, trolox equivalent antioxidant capacity and reducing power of the extract by determining total antioxidant capacity of the extract. The DPPH and TEAC assays are based on the ability of DPPH or ABTS radicals respectively, to decolorize when accept an electron donated by antioxidant compounds [21]. The antioxidant activity reported in the Table 1. The extract was capable of scavenging the DPPH radical in a concentration-dependent manner (Figure 1). The estimated EC_{50} of the methanolic extract was similar to that of (+)-catechin (111.2 ± 12.), used as the positive control. On the other hand the reducing ability of a compound generally depends on the presence of reductants [22], which have been exhibited antioxidative potential by breaking the free radical chain, donating a hydrogen atom [23]. The presence of reductants in *L.schottii* extract causes the reduction of the Fe^{3+}/ ferricyanide complex to the ferrous form. Therefore, the

Table 1. Radical Scavenging Activities of L. schottii



Data were expressed as the mean ± SD of three replicates.
*equivalent/1 g extract

Figure 1. Dose inhibition curve of the crude extract of L. schottii.



Data were expressed as the mean ± SD of three replicates.

Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 595 nm (Table 1). Based on the results described above, we conclude that the methanol extract of aerial parts of *L. shottii* shows strong antioxidant activity, and can be used as an easily accessible source of natural antioxidants. The components responsible for the antioxidant activity of the methanol extract are probably the phenolic compounds, particularly the flavonoids, which have been isolated from Cactaceae species, and may contribute directly to their antioxidative action [24]. It has been suggested that polyphenolic compounds have inhibitory effects against oxidative stress. The methanol extract of *L. schottii* is a promising source of antioxidants compounds. The isolation of bioactive compounds in the extracts would help to ascertain the individual potency of the compounds.

Acknowledgments

This study was financially supported by UANL-PAICYT-SA-999-04.

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