ORIGINAL ARTICLE

In vitro antioxidant activity of ethanolic extract of leaves of *cnidoscolus chayamansa* Mc Vaugh (Euphorbiaceae)

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Abstract

Background: This study was aimed to investigate the antioxidant activities of the ethanolic extract of leaves of *Cnidoscolus chayamansa* Mc Vaugh (Euphorbiaceae). **Materials and Methods:** The antioxidant activities of extract have been evaluated using a range of *in vitro* assays. In these studies, the percentage inhibition values were found to be in 2, 2-diphenyl-1-picrylhydrazyl (DPPH), nitric oxide (NO), hydroxyl radical scavenging assays, respectively. **Results and Discussion:** The results were analyzed statistically by the regression method. Its antioxidant activity was estimated by inhibitory concentration value, and the values are 262.02 µg/ml (DPPH radical scavenging), 343.01 µg/ml (NO scavenging), and 224.47 µg/ml (hydroxyl radical scavenging activity). In all the testing, a significant correlation exists between concentrations of the extract and percentage inhibition of free radicals. The antioxidant property may be related to the polyphenol and flavonoids present in the extract. **Conclusion:** This study on *in vitro* antioxidant activities of *C. chayamansa*, which justifies the ethnomedical use of this plant.

Key words: Antioxidant activity, *Cnidoscolus chayamansa,* phytoconstituents

INTRODUCTION

Antioxidants scavenge free radicals which are associated with various physiological and pathological events such as inflammation, aging, mutagenicity, and carcinogenicity. Any food or drug needs to claim some antioxidant property. The tree spinach *Cnidoscolus chayamansa* Mc Vaugh, (Euphorbiaceae), called "*Chaya*" in South Texas, is popular in Mexico and central America and has been introduced into the United States (Mainly South Texas and Florida) and now presently available in and around Southern part of India, for potential uses as a leafy vegetable and or as a medicinal plant. The edible parts of *C. chayamansa* plant which taste such as spinach when cooked, provide important nutritional sources for proteins, vitamins (A and C), minerals (calcium, iron, phosphorus), niacin, riboflavin, and thiamine. Among populations that cannot afford expensive foods rich in these nutrients.[1] *C. chayamansa* traditionally has been recommended for a number of ailments including diabetes, obesity, kidney stones, hemorrhoids, acne and eye problems.[2]

C. chayamansa shoots and leaves have been used as a laxative, diuretic, circulation stimulant to improve digestion to stimulate lactation and to harden the finger nails.[3] The leaves contain mineral constituents such as K, Ca, Mg, Na, Fe, Mn, Zn, and Cu, flavonoids such as amentoflavone, Astragatin, kaempferol-3o-ruttinoside and dihydromyricetin. Leaves also contain hydrocyanic glycosides, a toxic compound easily destroyed by cooking, even though some people tend to eat raw *C. chayamansa* leaves, it is unwise to do so while the nutritional value of *C. chayamansa* has been demonstrated.[4] Based on the above background this study was aimed to demonstrate the antioxidant potential of ethanolic extract of *C. chayamansa* leaves.

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MATERIALS AND METHODS

Plant Collection and Extraction

The leaves of *C. chayamansa* Mc Vaugh was collected from in and around Kanyakumari District, Tamil Nadu. The leaves of the plant *C. chayamansa* were dried in the shade, milled into coarse powder by a mechanical grinder and packed into Soxhlet apparatus and extracted with 70% v/v ethanol in water at 75-79°C for 22 h. The extract obtained was evaporated at 45°C, then dried and stored in airtight container. The yield of the extract was 24.8% w/w.

Determination of Total Phenolic Contents

The total phenolic content of the sample was determined according to the method described by Siddhuraju and Becker.[5] 10 µl aliquots of the extracts (1 mg/ml) were taken in test tubes and made up to the volume of one ml with distilled water. Then 0.5 ml of Folin–Ciocalteu phenol reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in the dark for 40 min, and the absorbance was recorded at 725 nm against the reagent blank. The analysis was performed in triplicate, and the results were expressed as tannic acid equivalents (TAE).

Determination of Total Flavonoid Content

The flavonoid content of the sample was determined by the use of a slightly modified colorimetry method described previously by Zhishen *et al*.[6] A 0.5 ml aliquot of appropriately (1 mg/ml) diluted sample solution was mixed with 2 ml of distilled water and subsequently with 0.15 ml of 5% $NaNO_2$ solution. After 6 min, 0.15 ml of 10% $AlCl_3$ solution was added and allowed to stand for 6 min, and then 2 ml of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5 ml, and then the mixture was thoroughly mixed and allowed to stand for another 15 min. The absorbance of the mixture was determined at 510 nm versus water blank. The analysis was performed in triplicate, and the results were expressed as rutin equivalent (RE).

Free Radical Scavenging Activity on 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH)

The antioxidant activity of the sample was determined in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH, according to the method of Blois.[7] The sample extracts at various concentrations (200-1000 µg) were taken, and the volume was adjusted to 100 µl with methanol. 5 ml of 0.1 mM methanolic solution of DPPH was added and allowed to stand for 20 min at 27°C. The absorbance of the sample was measured at 517 nm. Percentage radical scavenging activity of the sample was calculated as follows:

% DPPH radical scavenging activity = (control OD - sample OD/control OD) × 100

The analysis was performed in triplicate. The sample concentration providing 50% inhibition under the assay condition was calculated from the graph of inhibition percentage against sample concentration.

Hydroxyl Radical Scavenging Activity (HRSA)

The scavenging activity of the sample on hydroxyl radical was measured according to the method of Klein *et al*. (1981).[8] Different concentrations of the extract (200-1000 µg) were added with 1 ml of iron-ethylenediaminetetraacetic acid (EDTA) solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml of EDTA solution (0.018%), and 1 ml of dimethyl sulfoxide (0.85% v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5 ml of ascorbic acid (0.22%) and incubated at 80-90°C for 15 min in a water bath. After incubation, the reaction was terminated by the addition of 1 ml of ice-cold trichloroacetic acid (17.5% w/v). 3 mL of Nash reagent (75.0 g of ammonium acetate, 3 ml of glacial acetic acid, and 2 ml of acetyl acetone were mixed and raised to 1 L with distilled water) was added and left at room temperature for 15 min. The intensity of the color formed was measured spectroscopically at 412 nm against reagent blank. The percentage HRSA was calculated as follows:

% HRSA = (control OD - sample OD/control OD) × 100

The analysis was performed in triplicate. The sample concentration providing 50% inhibition concentration (IC_{50}) under the assay condition was calculated from the graph of inhibition percentage against sample concentration.

Nitric Oxide (NO) Radical Scavenging Activity

The NO scavenging activity of the sample was measured according to the method of Sreejayan and Rao.[9] 3 ml of 10 mM sodium nitroprusside in 0.2 M phosphate buffered saline (pH 7.4) was mixed with different concentrations (200-1000 µg) of solvent extracts and incubated at room temperature for 150 min. After incubation time, 0.5 ml of Griess reagent (1% sulfanilamide, 0.1% naphthy lethylene diamine dihydrochloride in 2% H_3PO_4) was added. The absorbance of the chromophore formed was read at 546 nm. The percentage radical scavenging activity of the sample was calculated as follows:

% NO radical scavenging activity = (control OD - sample OD/control OD) × 100

Table 1: Total phenolic and flavonoid content of ethanolic extract *C. chayamansa*

Sample	Total phenols (mg TAE/g extract)	Flavonoid (mg RE/g extract)
Ethanolic extract *C. chayamansa*	17.97±0.33	1.06±0.02

Values are means of three independent analyses of the extract±SD (*n*=3), SD: Standard deviation, *C. chayamansa*: *Cnidoscolus chayamansa,* TAE: Tannic acid equivalents, RE: Rutin equivalent

Table 2: Comparison of IC_{50} values of extract with standard for *in vitro* antioxidant activity

Model	IC_{50} value of EECC (µg/ml)	IC_{50} value of standard (µg/ml)
DPPH radical scavenging activity	262.02±1.97	1.578±0.03
NO radical scavenging activity	343.01±8.91	6.26±0.18
HRSA	224.47±5.43	17.25±0.23

Values are means of three independent analyses±SD (*n*=3), SD: Standard deviation, DPPH: 2, 2-diphenyl-1-picrylhydrazyl, NO: Nitric oxide, HRSA: Hydroxyl radical scavenging activity, IC_{50}: Inhibition concentration

The analysis was performed in triplicate. The sample concentration providing IC_{50} under the assay condition was calculated from the graph of inhibition percentage against sample concentration.

RESULTS AND DISCUSSION

Phenolic and flavonoidal substances are reported to be responsible for the biological activities of plant extracts.[10] The total phenol and flavonoid contents of the ethanolic extract were investigated and found to be 17.97 ± 0.33 mg TAE equivalent/g extract and 1.06 ± 0.02 mg RE equivalent/g extract, respectively, which was presented in Table 1. Phenolic content was observed rich compared to flavonoid content. Still, *C. chayamansa* was reported to contain flavonols such as kaempferol and quercetin in both raw and cooked leaves.[11] Several concentrations ranging from 200 to 1000 µg/ml of the ethanolic extract of *C. chayamansa* were tested for their antioxidant activity in different *in vitro* models. It was observed that free radicals were scavenged by the test compounds in a concentration dependent manner up to the given concentration in all the models. The percentage scavenging and IC_{50} values were calculated for all models given in Table 2. Free radicals play an important role in chronic diseases related to oxidative stress, such as diabetes, cancer, and cardiovascular pathologies.[12] Therefore, the free radical scavenging properties of the extract were determined by the DPPH, NO, and hydroxyl radical scavenging assays. Figure 1 shows the scavenging ability of equivalent iron chelation capacity against DPPH, NO, and hydroxyl radicals. The activity was dose-dependent and the IC_{50} were 262.02 ± 1.97, 343.01 ± 8.91, and 224.47 ± 5.43 for DPPH, NO, and hydroxyl radical, respectively. Maximum activity for DPPH assay was reported (71.90 ± 0.71) in 1000 µg/ml concentration of extract. Comparing to DPPH, less activity was reported in scavenging NO (58.56 ± 1.03) and hydroxyl radical (61.67 ± 1.88). IC_{50} values were low compared to the standard buthylated hydroxyanisol tested.

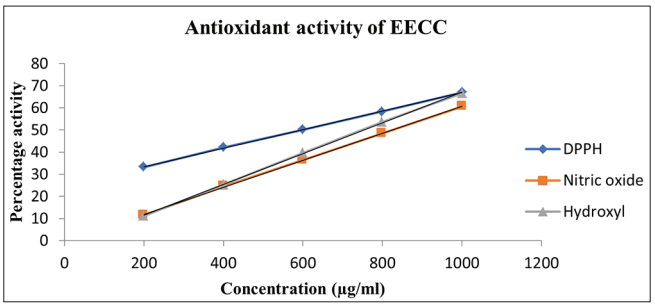

Figure 1: Scavenging ability of equivalent iron chelation capacity against 2,2-diphenyl-1-picrylhydrazyl, nitric oxide, and hydroxyl radicals

CONCLUSION

It can be concluded from our experimental results that the ethanolic extract of leaves from *C. chayamansa* showed a high content of phenolic compounds and flavonoids content and exhibits the greatest antioxidant activity through the scavenging of free radicals which participate in the various pathophysiology of diseases including aging. The extract was capable of scavenging DPPH, NO, and hydroxyl radicals in a concentration-dependent manner.

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