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Research Article

ESTIMATION OF TOTAL PHENOLIC CONTENT AND EVALUATION OF *IN-VITRO* ANTIOXIDANT ACTIVITY *CAPSICUM ANNUM* LINN. LEAVES

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Abstract: Methanolic extract of dried leaves of *Capsicum annum* L. was studied for its phytochemical properties. The extract revealed the presence of alkaloids, steroids, tannins, phenolic compounds, flavanoids, saponins and proteins. The total phenolic content was evaluated for the extract and it was further subjected to *in- vitro* anti-oxidant activity. The results were found to be significant.
Key**words:** *Capsicum annum* leaves, total phenolic content, antioxidant, phytochemical studies

INTRODUCTION

The genus Capsicum belongs to the Nightshade family which comprises of many other edible plants such as potatoes, tomatoes, eggplants. There are about 25 known wild varieties, though most cultivated chilli peppers are the varieties of the annum species.[1] It is an edible fruit belonging to the Solanaceae family. It is famous for its intense bitterness and heat produced after its consumption. Capsaicin is the substance found in the fruit which is responsible for its hot and spicy flavour. Green chilly is having a large number of synonyms such as in Hindi - Hari Mirch, Punjabi - mirch, Assamese - bhojolok, Bengali-lonkamorich, Telegu - Mirapa kaya, etc.[2,3,4,5] Traditionally, this food plant is used as a remedy for the health conditions or effects [6]: such as Abscesses, Boils, Furuncles, Menstrual cramps (dysmenorrhea),Skin infections ,Sores. The leaves are prepared by heating them and applying them to the affected area. Chilli peppers, the fruits of this plant, are considered hot and spicy condiments that are said to warm the body and are often added to nutritious, healing soups [7]. Chilli consists of chemical constituents such as capsaicinoids, carotenoids, Vitamin A, Vitamin C, minerals, steroids, steroidal glycosides, polyphenols and macronutrients.[2, 8 , 9, 10] Medicinally, it is used as a natural pain killer.[11,12,13,14,15,16,17] Green chilly is used to lower the blood cholesterol level, triglyceride levels, blood pressure and heart rate.[18,19,20] It shows strong antioxidant activity.[9] Its juice also posses strong antibacterial and antifungal activity.[2,6,21] The majority of published scientific literature on this plant has focused on fruit. None of the studies identified evaluated the biological activity of the leaves (the part of the plant most commonly used by Dominicans in New York City); instead, available research focuses on extracts of the fruit.[6] So, we have chosen leaves of *Capsicum annum* for our evaluation.

MATERIALS AND METHODS

Plant material (*Capsicum annum* L) were collected from Kodad, Andhra Pradesh, India, and authenticated by Dr. K. Madhavi Chetty, Asst. Prof., Department of Botany, Sri Venkateswara University, Tirupathi. The leaves were collected, shade dried and coarsely powdered. 100g g of the powdered sample is extracted with 1L of methanol (A.R.) in Soxhlet Apparatus. The extract was then evaporated and dried. The extract was evaluated for its phytochemical constituents. The Total Phenolic Content of the extract was determined and then subjected to *in-vitro* Antioxidant activity. The antioxidant activity was evaluated by its free radical-scavenging activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and Reducing Power assay (Fe^{3+}).

Chemicals and Reagents

Gallic acid (GA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin–Ciocalteu's phenol reagent were purchased from Sigma Chemical Company (St. Louis, MO, USA), Potassium ferricyanide, Trichloroacetic acid, Ferric Chloride, Ascorbic Acid and all solvents used were of analytical grade. They are purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Visible spectra measurements were done using UV-3000 spectrophotometer (LABINDIA, India).
Phytochemical Studies:
Qualitative analytical [22] tests were also carried out; alkaloids, saponins, flavonoids, polyphenols are present. All the chemicals and reagents used were of analytical grade. Results are given below in Table No.1.

THERAPEUTIC ACTIVITIES

Total Phenolic Content [23, 24]

The Total Phenolic Content in the extracts was determined by using Folin–Ciocalteu's phenol reagent. Aliquots of the extracts were taken in a 10 ml glass tubes. Then 9 ml of Distilled Water and 1 mL of Folin–Ciocalteu's phenol reagent (1:1 with water) were added and the contents mixed thoroughly. After 5 min, 10 ml of sodium carbonate (7 %w/v) were added sequentially in each tube. A blue color was developed in each tube because the phenols undergo a complex redox reaction with phosphomolibdic acid in folin

ciocalteau reagent in alkaline medium which resulted in a blue colored complex, molybdenum blue.

A standard calibration plot was generated (**Figure-1**) at 750 nm using known concentrations of Gallic Acid. The concentrations of phenols in the test samples were calculated from the calibration plot and expressed as mg Gallic Acid equivalent of phenol/g of sample. The mixed solution was then immediately diluted to required volume (25 ml) with deionized distilled water and mixed thoroughly. The tubes were allowed to stand for 90 min before absorbance at 750 nm was measured by using UV-3000 spectrophotometer (Lab India). The TPC were calculated by using GA calibration curve. The calibration equation for GA was $Y = 0.07411X + 0.0589$ ($R^2 = 0.9977$).

In-Vitro Antioxidant Activity [25, 26]

Antioxidant activity of the extracts was evaluated by DPPH free radical-scavenging capacity and Reducing Power method.

DPPH free radical-scavenging capacity [27,28,29,30,31]

Antioxidants react with DPPH, which is a stable free radical and is reduced to the DPPHH and as consequence the absorbance's decreased from the DPPH radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability. [32]

Procedure:

The ability of the plant extract to scavenge 1,1-diphenyl-2-picryhydrazyl (DPPH) free radicals was assessed by the standard method. The stock solution of extracts were prepared in ethanol to achieve the concentration of 1 mg/ml. Dilutions were made to obtain concentrations of 50, 100, 250, 500 µg/ml. Diluted solutions (1 ml each) were mixed with 3 ml of ethanolic solution of DPPH (DPPH, 0.004%). After 30 min of incubation at room temperature the reduction of the DPPH free radical was measured by reading the absorbance at 517nm using UV-Visible Spectrophotometer DPPH is a purple colored stable free radical; when reduced it becomes the yellow-colored diphenyl-picryl hydrazine. Initially, absorption of blank sample containing the same amount of ethanol and DPPH solution was prepared and measured as control. Ascorbic acid was used as standard. The experiment was carried out in triplicate. Percentage inhibition was calculated using the following equation, whilst IC_{50} values were estimated from the % inhibition versus concentration plot, using a non-linear regression algorithm. The data were presented as mean values ± standard deviation (n = 3) in Table no.2

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Sample})}{\text{Absorbance of control}} \times 100$$

Ferric ions (Fe^{3+}) reducing power assay [33, 34]

Procedure:

10 mg of extract in 1ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN_6]$ (2.5 ml, 1%). The mixture was incubated at 50◦C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 RPM for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm using Digital Photo Colorimetry (Ri, India, Sl. No. 09/9/441). Ascorbic acid was used as the reference material. All the tests were performed in triplicate and the graph was plotted with the average of three observations.

Statistical analysis

Results were given as mean ± standard deviation of 3 replicates. Differences between means were determined using one-way ANOVA and Duncan's test. The level of statistical significance was set at $P \leq 0.0001$

RESULTS AND DISCUSSIONS

Phytochemical Screening

The extract was subjected to qualitative preliminary phytochemical test. All the chemical tests are done and reported accordingly in Table No.1. The table indicates the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins, proteins and steroids. The presence of flavonoids, phenolic compounds might be responsible for its *in-vitro* antioxidant, anti-Inflammatory and thrombolytic activity.

Table No. 1: Phytochemical analysis of different *C. annum* leaves extracts

Sl. No.	Phytoconstitutents	Test
1.	Alkaloids	+
2.	Protein	+
3.	Carbohydrate	-
4.	Reducing sugar	-
5.	Tannins	+
6.	Saponins	+
7.	Terpenoids	-
8.	Glycosides	-
9.	Flavanoids	+
10.	Phenolics	+
11.	Volatile oil	-
12.	Steroids	+

+ = present; - = absent

Total Phenolic Contents

Polyphenols are a large and diverse class of compounds, many of which occur naturally in a wide range of food and plants. The flavonoids are the largest and best studied group among polyphenols. A range of plant polyphenols is either being actively developed or already currently sold as dietary supplements and/or herbal, derived medicines. Although these compounds play an unknown role in nutrition (non-nutrients), many of them have properties including antioxidant, anti-mutagenic, anti-carcinogenic and anti-inflammatory effects that might potentially be beneficial in preventing disease and protecting the stability of genome.[36] Antioxidant quality is a measure of the effectiveness of the antioxidant(s) present as a pure compound or a mixture. [37] The percentage scavenging and IC_{50} values were calculated for all models. Phenolic compounds are known to be powerful chain breaking antioxidants and are important constituents of plants. Phenolic compounds may contribute directly to antioxidative action. It is suggested that phenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when ingested up to 1.0 gm daily from a diet rich in fruits and vegetables. The total phenolic content of the extract of *C. annum* was measured by Folin-Ciocalteu reagent in terms of gallic acid equivalent (GAE)/g of dry sample (standard plot: y = 0.007x + 0.056, R^2 =0.995). The Standard graph is shown in Figure 2.The values were found to be 33.42 mg Gallic Acid equivalent /g. Phenolics present in the leaves, have received considerable attention because of their potential biological activities of the fruits. Results are in Table No. 2.

Table No. 2: Total Phenolic contents of *C. annum* leaf extracts

Concentration	Total Phenolic Content (mg/g GAE)
800(ug/ml)	33.42±0.13

Values are in Mean ±SD for three readings

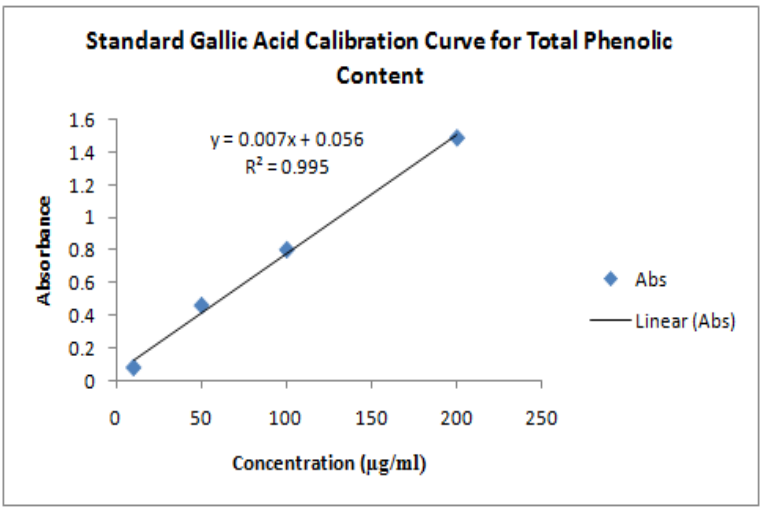

Figure-1: Standard Gallic Acid curve for estimation of Total Phenolic content

***IN- VITRO* ANTIOXIDANT ACTIVITY**

DPPH Free Radical - Scavenging Capacity

The reactivity of methanolic extract of *C. annum* extract was analyzed with DPPH, a stable free radical. Polyphenolic compounds present in *C. annum* contributes significantly to the total antioxidant capacity of the leaves. The DPPH radical scavenging (%) activity is shown in the Fig 5. In the present study, reduction of the DPPH radicals was found in concentration- dependent manner. The *C. annum* methanolic extract reduced the stable DPPH radical to yellow colored unstable compound, with an IC_{50} value of (**91.2**µg/ml). However, ascorbic acid displays significant scavenging activity over the leaves methanolic extract. The scavenging activity was found to be highest at 500 µg/ml with 80% inhibition for the leaves, while for the Ascorbic acid, the % DPPH scavenging activity was 80% at 100µg/ml. The decrease in the absorbance indicates the increase in the scavenging activity. As DPPH picks up one electron in the presence of a free radical scavenger, the absorption decreases and the resulting discoloration is stochiometrically related to the number of electrons gained.[38, 39] When the results of the extract are compared with the control, the % inhibition values were found to be significant, (P<0.0001). All the statistical data are presented in Table No. 3

Table No.3: DDPH scavenging activity of *C. annum* leaf extracts

Sl. No.	Concentration (µg/ml)	% Scavenging Activity (Mean±SD)	IC_{50} (µg/ml)
Std (Ascorbic Acid)			
1	12.5	27.41± 2.46***	39.85
2	25	45.19± 1.61***	
3	50	62.9± 1.62***	
4	100	80.64± 2.46***	
TEST			
1	100	47.6 ± 2.46***	91.2
2	200	62.5 ± 2.45***	
3	500	80 ± 1.65***	

Values are expressed as Mean ± SD, in triplicate results; ***P<0.0001

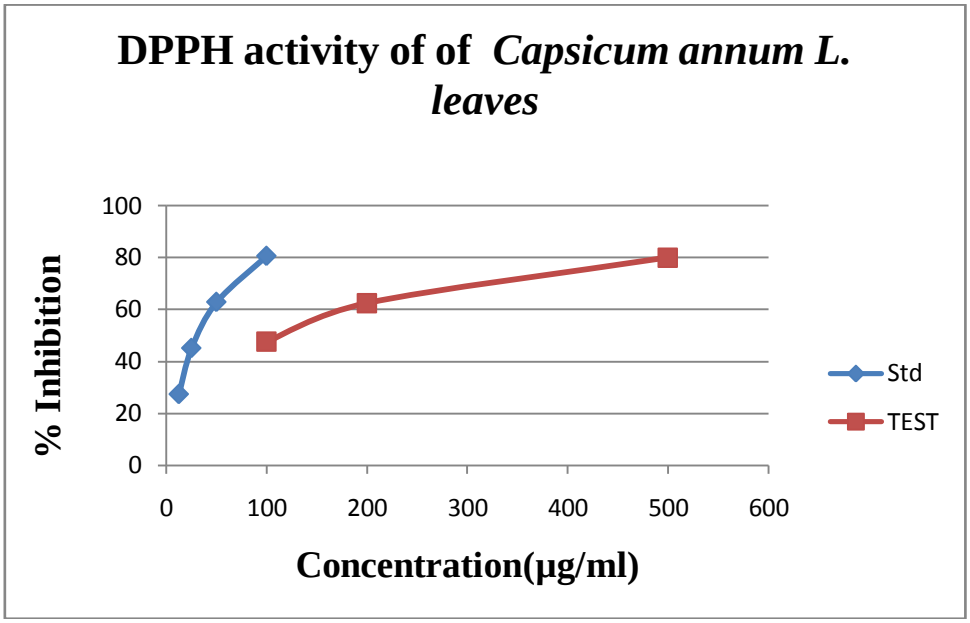

Figure: 2- DDPH scavenging activity of *C. annum* leaf extracts

Ferric Reducing Power Assay

The methanolic extract exhibited dose –dependent increase in the reducing power activity of Ferric ions. The results are displayed in Table no. 4 and graph in figure 3. It showed the highest reducing activity at 500µg/ml with 1.18 ± 0.015 absorbance. The reducing power of a compound may serve as a significant indicator of its potent antioxidant activity. The antioxidant can donate an electron to free radicals, which leads to the neutralization of the radical. Reducing power was measured by direct electron donation in the reduction of Fe^{3+} $(CN^{-})_6$ –Fe^{2+} $(CN^{-})_6$ [40] The product was visualized by forming the intense Prussian blue color complex and then measured at λ700nm. As shown in Fig. 3, a higher absorbance value indicates a stronger reducing power of the samples. *C. annum* extract showed concentration- dependent reducing power. However, its reducing power was weaker than that of Ascorbic acid, which exhibited the strongest reducing power. Antioxidant compounds are able to donate electrons to reactive radicals, reducing them into more stable and unreactive species.[41]

Table No.4: Reducing power activity of *C. annum* leaves extracts

Sl. No.	Concentration (µg/ml)	Absorbance			Absorbance (Mean±SD)
		1	2	3	
Std (Ascorbic Acid)					
1	12.5	0.23	0.21	0.22	0.22± 0.01
2	25	0.48	0.47	0.46	0.47±0.01
3	100	0.78	0.76	0.77	0.77±0.01
4	200	0.22	1.2	1.19	0.20±0.015
TEST					
1	100	0.45	0.46	0.44	0.45±0.01
2	200	0.88	0.87	0.90	0.88±0.015
3	500	1.2	0.18	1.17	.18±0.015

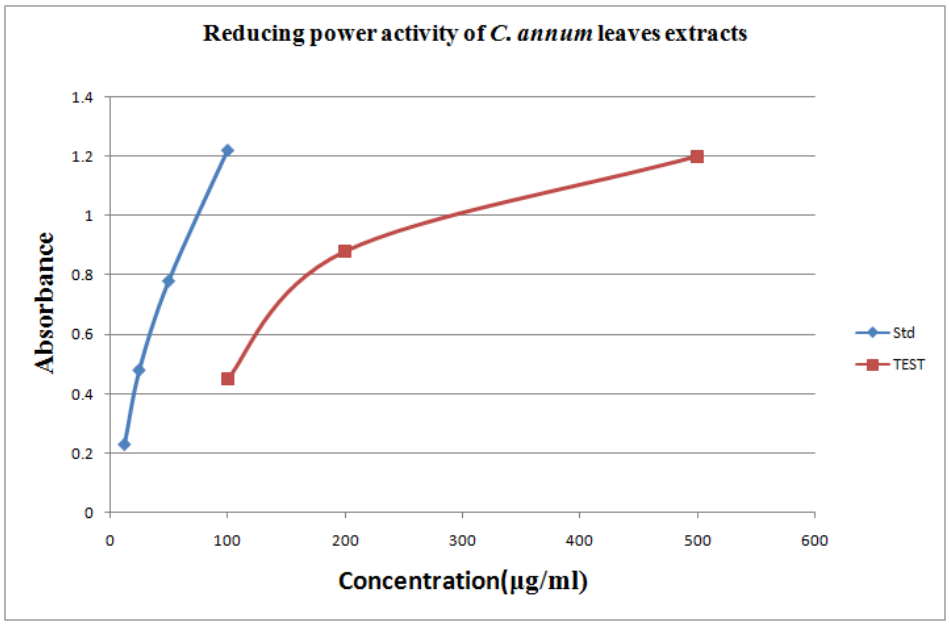

Figure -3: Reducing power activity of *C. annum* leaves extracts

Conclusion

As a dual purpose plant, for its medicinal and edible purpose, *C. annum* leaves are used for skin conditions and dermatological infections, the leaves are heated and applied externally to the affected area as a poultice. For menstrual cramps and symptoms related to dysmenorrhea, the leaves are prepared as a tea by decoction or infusion. It was extensively used to treat diabetics in folk medicine. Saponins, flavonoids, polyphenols, alkaloids are the bioactive constituents of the plant. We have herein provided the available information about the antioxidant activities of the methanol extract of *capsicum* leaves. The results emphasize the extract of *C. annum* has possessed significant antioxidant properties. Therefore, *C. annum* would be a potential source of natural antioxidants and nourishment. The consumption of leaves in the might give positive function of health protection against oxidative damages. With the ascertained antioxidant activity of this plant, the separation and identification of the antioxidative components in the methanol extract and using response–surface methodology (RSM) optimization, the extraction of phenolic compounds should be further investigated.

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