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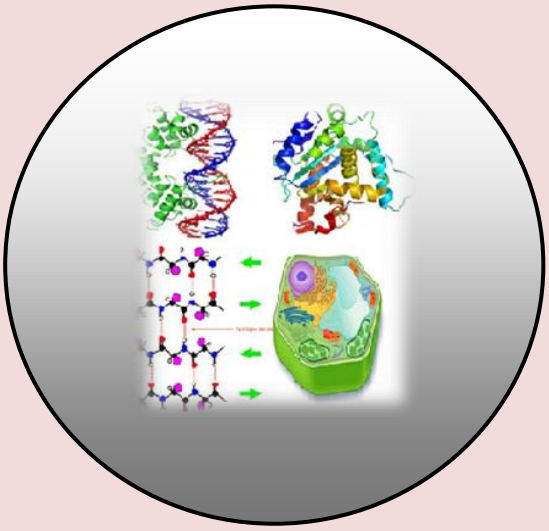
ISSN 2319-3077 Online/Electronic
ISSN 0970-4973 Print

UGC Approved Journal No. 62923
MCI Validated Journal
Index Copernicus International Value
IC Value of Journal 82.43 Poland, Europe (2016)
Journal Impact Factor: 4.275
Global Impact factor of Journal: 0.876
Scientific Journals Impact Factor: 3.285
InfoBase Impact Factor: 3.66

J. Biol. Chem. Research
Volume 36 (1) 2019 Pages No. 184-197



Journal of Biological and Chemical Research

An International Peer Reviewed / Referred Journal of Life Sciences and Chemistry

Indexed, Abstracted and Cited in various International and National Scientific Databases

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 36, No. 1: 184-197, 2019
(An International Peer Reviewed / Refereed Journal of Life Sciences and Chemistry)
Ms 36/01/89/2019

ISSN 2319-3077 (Online/Electronic)
ISSN 0970-4973 (Print)

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http:// www.sasjournals.com
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RESEARCH PAPER
Received: 25/12/2018 **Revised: 24/01/2019** **Accepted: 25/01/2019**

Antioxidant Activities, Thin Layer Chromatographic Analysis and GCMS Analysis of *Capsicum annuum* L.: A Comparison of Green and Red Chilli

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ABSTRACT

Chilli pepper (Capsicum annuum L.) is a widely cultivated species that has been used since ancient times as food flavoring and for human health. Capsaicin is currently used for the treatment of diabetic neuropathy, osteoarthritis, post-herpetic neuralgia and psoriasis as well as pesticides, insecticides, insect or animal repellentscontaining capsaicinoids. The aim of the present study was to evaluate the antioxidant activity and to identify the bioactive compounds by performing GC-MS analysis comparatively for Red and Green Chilli. The $IC_{50}$of DPPH$^{\cdot}$radical, ABTS$^{\bullet+}$radical cation, Superoxide ($O_2^{\cdot-}$) radical scavenging assays were 34.24, 9.85 and 37.18 µg/mL concentration respectively. The RC_{50} of phosphomolybdenum reduction and Fe3+ reduction assays were 20.22 and 41.54 µg/mL concentration respectively. The GCMS analysis revealed the presence of active compounds such as Coumarine,3-[2-(1-methyl-2-imidazolylthio)-1-oxoethyl]-,Piperidine-2-carboxamide,1-amino-N-mesityl, Phytol, Flavone and Oleic acid exhibiting various biological activities such as antioxidant, anticancer, antimicrobial, etc. The results of this study portray the effective antioxidant activity of C. annuum and further studies are required to isolate the active compounds from various parts of this species and their mode of action.

Keywords: Capsicum annuum, Antioxidant, Dot-plot, DPPH, Superoxide ($O_2^{\cdot-}$) Radical and GCMS.

INTRODUCTION

Capsicum annuum is a domesticated species of the plant genus Capsicum in the family of Solanaceae (Greenleaf, 1986) native to southern North America and northern South America. The fruit is a berry and may be green, yellow, or red when ripe (Zhang et al. 2002). There are more than 200 common names in use for this species.Chillies were cultivated around the globe after Columbus (Eshbaugh, 1993). From Mexico, at the time the Spanish colony that controlled commerce with Asia, chilli peppers spread rapidly into the Philippines and then to India, China, Indonesia, Korea and Japan. Chilli fruits are of various shapes and size which depend upon the commercial variety ofthe drug. In general it is oblong, conical, 10-20 mm long and 4-7 mm wide. In the glabrous pericarp is attached to cup-shaped five toothed calyx and straight pedicel. Pericarp is orangered to dark reddish, shrunken and thin. Internally the pericarp is divided into two cells by the membranous dissepiment. Each cell consist of about 5-10 small, flattened, disc shaped whitish yellow seeds (Rangari and Vinod, 2006).

MATERIALS AND METHODS

Collection of chilli fruits andpreparation of extracts

The red and green chilli fruits of *C. annuum* were bought from the vegetable market at Maduvinkarai, Guindy, Chennai. The collected chilli fruits were washed with tap water, cut into pieces and soaked in ethanolfor 72 h at room temperature. Then the supernatant was filtered by filter paper and condensed by using rotary evaporator 50°C, which yields gummy extract (Trease and Evans, 1983; Harborne, 1998).

Taxonomy

Kingdom: Plantae – Plants
Subkingdom: Tracheobionta – Vascular plants
Superdivision: Spermatophyta – Seed plants
Division: Magnoliophyta – Flowering plants
Class: Magnoliopsida – Dicotyledons
Subclass: Asteridae
Order: Solanales
Family: Solanaceae – Potato family
Genus:*Capsicum* L. – pepper P
species:*Capsicum annuum* L.

Figure 1. Habitat of red and green Chilli of *C.annuum.*

The Active component, capsaicin has protective effect on respiration process, anti-genotoxic and anti-carcinogenic property and it's a dietary phyto-constituent molecule with effective chemo-preventive activity. Capsaicin compound has the ability to block the transmission pain to spinal cord from skin (Rangari and Vinod, 2006).

Screening of radical scavenging activity by dot-blot DPPH staining method

Drops of DPPH (0.4 mM) solution in methanol were loaded onto a 5 × 5 cm TLC plate (silica gel 60 F254; Merck) in each column and allowed to dry for 2 min. The first row of TLC plate was considered as control, containing only DPPH. Various concentrations (2-10µg/mL) of ethanol extracts of Red and Green chilli were carefully loaded onto the DPPH spot in the second and third rows successfully. The fourth row of TLC plate was considered as standard reference, where ascorbic acid was carefully loaded onto the DPPH spot (Soler-Rivas et al. 2000). Stained silica gel layer revealed purple background with yellow or white spots at the location where radical scavenging capacity observed.

Qualitative phytochemical analysis of red and green Chilli

Screening of phytochemicals for ethanol extracts of red and green chilli was carried out comparatively using standardized methods (Trease and Evans, 1983; Harborne, 1998).

Quantitative estimations of total phenol and flavonoids

Determination of total phenols

Folin-Ciocalteau reagent method was used to determine the total phenolic compounds (Spanos and Wrosltad, 1990) with slight modifications. One hundred µL of ethanol extracts (1mg/mL) of red and green chilli fruits were mixed with 900 µL of ethanol and 1 mL of Folin Ciocalteau reagent (1:10 diluted with distilled water). After 5 min, 1 mL of Na_2CO_3 (20% w/v) solution was added.

The mixture was then allowed to stand for 30 min incubation in dark at room temperature. The absorbance was measured by UV-Vis spectrophotometer at 765 nm. The total phenolic content was expressed in terms of gallic acid equivalent (µg/mg of extract), which is a common reference compound.

Determination of total flavonoids

The total flavonoid content of ethanol extracts (1mg/mL) of red andgreen chilli fruits were determined by aluminium chloride reagent method with slight modification (Liu et al. 2007). Five hundred µL of red and green chilli extracts (1mg/mL) were mixed with 0.5 mL of ethanol and 0.5 mL of (5% w/v) sodium nitrite solution. Then, 0.5 mL (10% w/v) aluminium chloride solution was added followed by 1 mL of 1 M NaOH solution was added. The mixture was incubated for 30 min at room temperature and the absorbance was measured at 510 nm. The result was expressed as (µg/mg of extract) quercetin equivalent.

Antioxidant activities

DPPH$^{\bullet}$ radical scavenging activity

The antioxidant activity of ethanol extracts of red and green chilli fruits of *C .annuum* was measured on the basis of scavenging activity of the stable 1, 1- diphenyl 2-picrylhydrazyl (DPPH) free radical (Blois, 1958). One mL of 0.1 mM DPPH solution in methanol was mixed with 1 mL of various concentrations (20-120 µg/mL)red and green chilli extracts. The mixture was then allowed to stand for 30 min incubation in dark. Ascorbic acid was used as the reference standard. One mL ethanol and 1mL of DPPH solution was used as the control. The decrease in absorbance was measured using UV-Visspectrophotometer at 517 nm. The percentage of inhibition was calculated as:

$$\%\ \text{of DPPH}^{\bullet}\ \text{radical inhibition} = \left(\frac{\text{Control–Sample}}{\text{Control}}\right)*100$$

ABTS$^{\bullet+}$radical cation scavenging activity

The antioxidant capacity was determined in terms of the ABTS$^{\bullet+}$ radical cation scavenging activity by the method (Delgado-Andrade and Morales, 2005). ABTS$^{\bullet+}$radical cationwas obtained by reacting 7 mM ABTS solution in water with 2.45 mM potassium persulfate and the mixture was left to stand in the dark at room temperature for 12-16 hours before use. The ABTS$^{\bullet+}$ solution was diluted with distilled water and set to reach an absorbance of 0.70±0.02 at 734 nm. OnemL of various concentrations(5-30µg/mL) of red and green chilli extractswere mixed with 500 µL of diluted ABTS$^{\bullet+}$radical cationsolution. After 10 min, the absorbance was measured at734 nm and the ABTS$^{\bullet+}$ radical cation scavenging activity was expressed as:

$$\%\ \text{of ABTS}^{\bullet+}\text{radical cation inhibition} = \left(\frac{\text{Control – Sample}}{\text{Control}}\right)*100$$

Superoxide ($O_2^{\cdot-}$) radical scavenging activity

The superoxide ($O_2^{\cdot-}$)radical scavenging activityof red and green chilli extracts of *C .annuum* was measured on the basis of superoxide radical inhibition(Lokesh Deb et al. 2009). One mL of various concentrations (10-60 µg/mL) of red and green chilli extracts were mixed with 50mM phosphate buffer (pH-7.6). The mixtures were combined with 200µL of 20µg Riboflavin, 200µL of 12mM EDTA followed by 100µL of NBT (1mg/mL). The reaction was started by illuminating the test tubes in UV-lamp for 10min. The superoxide ($O_2^{\cdot-}$) radical reduces NBT to a blue colored formazon can be measured at 590nm. The percentage of superoxide ($O_2^{\cdot-}$) radical inhibition can be calculated as:

$$\%\ \text{of superoxide } (O_2^{\cdot-})\text{radical inhibition} = \left(\frac{\text{Control – Sample}}{\text{Control}}\right)*100$$

Phosphomolybdenum reduction activity

The antioxidant capacity of theethanol extracts of red and green chilli fruits of *C.annuum*was assessed by the method (Prieto et al. 1999). The ethanol extracts of different concentrations (20-120 µg/mL) was combined with 1mL of reagent solutioncontaining 4mM Ammonium molybdate, 28mM Sodium phosphate and 0.6M Sulphuric acid. The reaction mixture was incubated in water bath at 95^{o}C for 90 min. The absorbance of the coloured complex was measured at 695 nm. Ascorbic acid was used as standard reference.

$$\% \text{ of phosphomolybdenum reduction} = \left[\frac{\text{Sample} - \text{Control}}{\text{Sample}} * 100\right]$$

Ferric (Fe^{3+}) reducing power activity

The reducing power of ethanol extracts of red and green chilli fruits of *C.annuum*was determined by slightly modified method of(Oyaizu, 1986). One mL of ethanol extracts of different concentrations (20 - 120 µg/mL) was mixed with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1 % w/v). The mixture was then incubated in water bath at 50°C for 30 min. One mL of trichloroacetic acid (10 % w/v) was added to each mixture, mixed well and 1mL of freshly prepared $FeCl_3$ (0.1% w/v) solution and shaken well.The absorbance was measured at 700 nm using spectrophotometer. Ascorbic acid was used as the standard reference.

$$\% \text{ of } Fe^{3+} \text{reduction} = \left[\frac{\text{Sample} - \text{Control}}{\text{Sample}} * 100\right]$$

Thin layer chromatography analysis

Thin layer chromatography (TLC) analysis was carried out forethanol extracts of red and greenchilli fruits of *C.annuum*onsilica gel aluminium sheet (Merck Silica gel 60 F254)(Stahl, 2005). The ethanol extracts were spotted at 0.5 mm above from the bottom of the TLC plate. The spotted TLC plate was placed in a 100mL beaker containing solvent mixture Toluene with the ratio of 2mL. The chromatogram was developed and the spots were visualizedunder UV light at 254 nm as well as in iodine vapour. The ratio in which distinct coloured bands appeared were optimized and R_f values were calculated.

$$R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Gas chromatography–Mass Spectrometry (GC–MS)

For GC-MS analysis, the samples were injected into a HP-5 column (30 m X 0.25 mm i.d with 0.25 µm film thickness), Agilent technologies 6890 N JEOL GC Mate II GC-MS model. Following chromatographic conditions were used: Helium as carrier gas, flow rate of 1 mL/min; and the injector was operated at 200°C and column oven temperature was programmed as50-250°C at a rate of 10°C/min injection mode. Following MS conditions were used: ionization voltage of 70 eV; ion source temperature of 250°C; interface temperature of 250°C; mass range of 50-600 mass units (Harini et al. 2017).

Identification of components

The database of National Institute Standard and Technology (NIST) having more than 62,000 patterns was used for the interpretation on mass spectrum of GC-MS. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

RESULTS AND DISCUSSION

Plant based drugs are considered as important sourcesfor therapeutic agents due to strong effect on curing several types of diseases with less side effects. The availability of modern medicines in today's World is of highly risk and more consumption leads to many side effects. Hence, medicinal plants derived drugs are effective for various treatments since they have low cost, easy available, less toxic in nature.Many herbs nearly 80-90% contain antioxidant rich compounds which protects the cells from free radicals. Free radicals once formed, it propagates chain reaction which leads to disorders such as aging, diabetes and cardiovascular diseases. Trapping and neutralizing the free radicals is the main role of antioxidant molecules which are present in the medicinal plants and nothing but the phytochemicals, such as phenols, flavonoids, tannins and terpenoids. Phenolic compounds and flavonoids are considered as potential source of antioxidant molecules when compared to other phyto-constituents due to it's hydrogen donating ability(Gennaro, 2000).

Screening of radical scavenging activity by dot-blot DPPH staining method

The results of dot-blot assay showed active spots in which various concentrations of ethanol extracts of red and green chilli were placed in respective rows. The zone exhibiting purple colour indicates that there is no antioxidant (free radical scavenging) activity and the zone exhibiting yellow colour indicates antioxidant activity. From the results obtained (Figure 2), it is evident that ethanol extract of green chilli has effective antioxidant activity, when compared to red chilli, also compared as well as standard ascorbic acid.

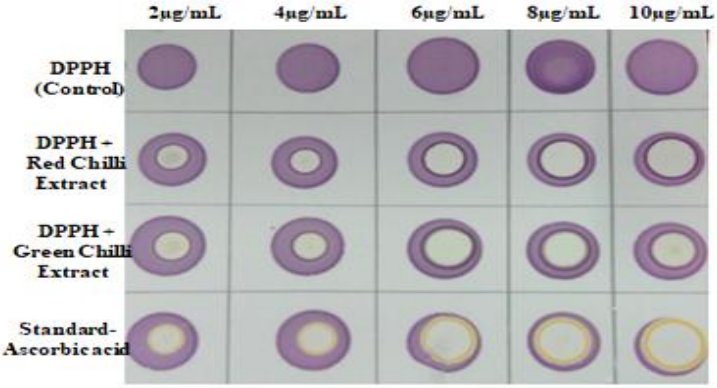

Figure 2. Dot-blot assay of ethanol extract of Red and Green Chilliof *C. annuum.*

Qualitative phytochemical analysis of red and green Chilli

The results of phytochemical analysis for ethanol extract of red chilli showed the presence of alkaloids, terpenoids, steroids. Comparatively, the ethanol extract of green chilli showed the presence of alkaloids. flavonoids, saponins, etc.

Table 1. Qualitative analysis of ethanol extract of fruits of Red Chilli and Green Chilliof *C. annuum.*

S.No	Phytoconstituents	Phytochemical Tests	Red Chilli-Results	Green Chilli-Results
1	Alkaloids	(a)Mayer's test	+	+
		(b)Hager's test	+	+
2	Phenols	Ferric chloride (5%) test	-	-
3	Tannins	Ferric chloride (0.1%) test	+	-
4	Flavonoids	Sodium hydroxide test	+	+
5	Glycosides	Legal's test	+	-
6	Steroids	Libermann-Burchard test	+	+
7	Terpenoids	Salkowski test	+	+
8	Saponins	Foam test	+	+

Quantitative estimations of total phenol and flavonoids

Determination of total phenols and flavonoids

Total phenol content for red chilli was 21.01±0.35 µg/mg of GAE and flavonoid content was 6.07±0.19 µg/mg of QE in the ethanol extract. Relatively, the total phenol content of ethanol extract of green chilli was 24.18±0.28 µg/mg of GAE and flavonoid content was 17.29±0.42 µg/mg of QE. Both phenols and flavonoids were found to be higher in ethanol extract of green chilli. Phenolic compounds and flavonoids have beneficial effects, which are mainly due to proper intake of healthy fruits and vegetables. Consumption of proper and nutritious foods might be responsible for significant antioxidant activity thereby preventing the entry of free radicals causing many diseases (Yen and Chen, 1995).

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Table 2. Quantitative estimation of ethanol extract of fruits of Red Chilli and Green Chilli of *C. annuum.*

S.No	Phytochemical	Value (µg/mg)*	
		Red Chilli	Green Chilli
1	Phenols	21.01±0.35 GAE	24.18±0.28 GAE
2	Flavonoids	6.07±0.19 QE	17.29±0.42 QE

(*Average value of 3 replicates)

Antioxidant activities

DPPH$^{\cdot}$ radical scavenging activity

Evaluation of antioxidant activity by DPPH method is the best screening option for herbal based drugs. The ability of the ethanol extract of red and green fruit of *C.annuum* to scavenge free radicals formed was assessed using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). The maximum DPPH$^{\cdot}$ radical scavenging activity was 88.34 % at 120µg/mL concentration for ethanol extract of green fruit of *C. annuum*. The DPPH$^{\cdot}$ radical scavenging activity was 33.40 % at 120µg/mL concentration for ethanol extract of red fruit of *C. annuum*. Thered and green fruit of *C.annuum* demonstrated high capacity for scavenging free radicals by reducing the stable DPPH (1,1-diphenyl-2- picrylhydrazyl) radical to the yellow coloured 1,1-diphenyl-2-picrylhydrazine and the reducing capacity increased with increasing concentration of the extract (Table 3). The IC_{50} value for ethanol extract of green fruit of *C.annum*was found to be 34.24µg/mL concentration (Figure 3) and was compared with standard (Ascorbic acid, IC_{50} = 11.98 µg/mL concentration).

Table 3. DPPH$^{\cdot}$ radical scavenging activity of ethanol extracts of green and red chilli of *C. annuum.*

S.No	Concentration (µg/mL)	% of inhibition*	
		Red Chilli	Green Chilli
1	20	14.12±0.43	27.57±0.39
2	40	18.07±0.26	58.40±0.25
3	60	21.30±0.22	81.61±0.12
4	80	27.80±0.18	84.75±0.48
5	100	32.73±0.20	86.32±0.27
6	120	33.40±0.36	88.34±0.16

(*Average value of 3 replicates)

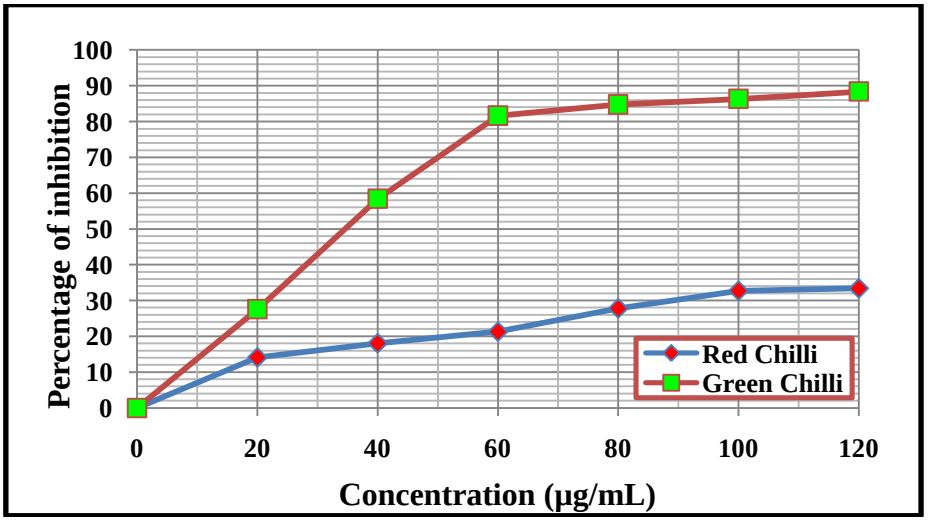


Figure 3. DPPH$^{\cdot}$ radical scavenging activity of ethanol extracts of green and red chilli of *C. annuum.*

Table 4. ABTS$^{\bullet+}$radical cation scavenging activity of ethanol extracts of green and red chilli of *C. annuum.*

S.No	Concentration (µg/mL)	% of inhibition*	
		Red Chilli	Green Chilli
1	5	33.81±0.36	40.64±0.25
2	10	47.84±0.27	50.71±0.11
3	15	59.35±0.38	62.58±0.36
4	20	69.06±0.40	66.90±0.42
5	25	83.09±0.17	85.25±0.29
6	30	87.05±0.35	89.56±0.14

(*Average value of 3 replicates)

ABTS$^{\bullet+}$radical cation scavenging activity

ABTS$^{\bullet+}$is a blue chromophore produced by the reaction between ABTS and potassium persulfate and in the presence of the plant extract or ascorbic acid, preformed cation radical gets reduced and the remaining radical cation concentration was then quantified. The maximum ABTS$^{\bullet+}$ radical cation scavenging activity of ethanol extract of green chilli of *C. annuum* was 89.56% at 30µg/mL concentration and 87.05% at 30µg/mL concentration for red chilli of *C. annuum*. (Table 4). The experiment demonstrated high antioxidant activity the IC_{50} of 9.85µg/mL concentration for ethanol extract of green fruit of *C.annuum* (Figure 4) and was compared with standard Ascorbic acid (IC_{50} = 4.21µg/mL concentration). The ABTS$^{\bullet+}$ radical is stable and solubility of ABTS$^{\bullet+}$ is with organic solvents and water, which determines the antioxidant capability of hydrophilic and lipophilic compounds.

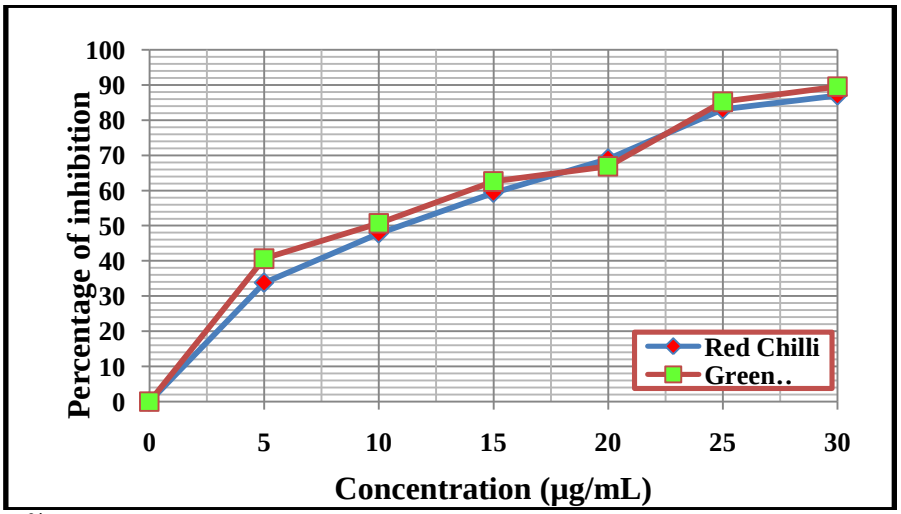


Figure 4. ABTS$^{\bullet+}$radical cation scavenging activity of ethanol extracts of green and red chilli of *C. annuum.*

Superoxide ($O_2^{\cdot-}$) radical scavenging activity

Reduction of NBT is the most popular method. The method is based on generation of super oxide radical by auto oxidation of riboflavin in presence of light. The maximum superoxide radical scavenging activity for ethanol extract of red chilli was 71.71% at 60µg/mL concentration and for ethanol extract of green chilli the superoxide radical scavenging activity was 76.01% at 60µg/mL concentration (Table 5). In-vitro super oxide radical scavenging activity is measured by riboflavin/light/NBT (Nitro blue tetrazolium) reduction. The experiment demonstrated higher antioxidant activity the IC_{50} of 37.18 µg/mL concentration for green chilli (Figure 5) and was compared with standard Ascorbic acid (IC_{50} = 4.54µg/mL concentration).

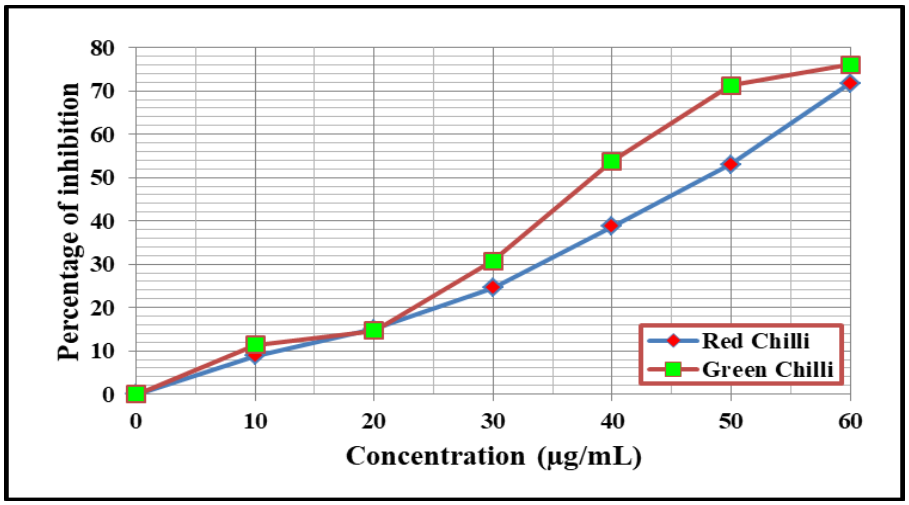


Figure 5. Superoxide ($O_2^{\cdot-}$) radical scavenging activity of ethanol extracts of green and red chilli of *C. annuum.*

Table 5. Superoxide ($O_2^{\cdot}$-) radical scavenging activity of ethanol extracts of green and red chilli of *C. annuum.*

S.No	Concentration (µg/mL)	% of inhibition*	
		Red Chilli	Green Chilli
1	10	8.82±0.43	11.36±0.18
2	20	15.06±0.28	14.64±0.42
3	30	24.49±0.46	30.80±0.14
4	40	38.73±0.38	53.78±0.40
5	50	53.03±0.24	71.21±0.26
6	60	71.71±0.17	76.01±0.39

(*Average value of 3 replicates)

Phosphomolybdenum reduction activity

The total antioxidant activity of ethanol extract of red and green fruit of *C.annuum*was measured spectrophotometrically by phophomolybdenum reduction method which is based on the reduction of Mo (VI) to Mo(V) by the formation of green phosphate/Mo (V) complex at acidic pH, with a maximum absorption at 695 nm. The maximum reducing ability for ethanol extract of red chilli was 78.57% at 120 µg/mL concentration and for green chilli, the reducing ability was comparatively higher as 90.73% at 120 µg/mL concentration (Figure 6). The experiment demonstrated higher antioxidant activity the RC_{50} of 20.22µg/mL concentration for ethanol extract of green chilli (Table 6) and was compared with standard Ascorbic acid (RC_{50} = 23.28µg/mL concentration).

Table 6. Phosphomolybdenum reduction activity of ethanol extracts of green and red chilli of *C. annuum.*

S.No	Concentration (µg/mL)	% of Phosphomolybdenum reduction*	
		Red Chilli	Green Chilli
1	20	31.7±0.26	49.45±0.21
2	40	39.78±0.18	74.07±0.38
3	60	48.62±0.39	81.39±0.40
4	80	68.53±0.11	87.3±0.18
5	100	71.71±0.30	90.08±0.41
6	120	78.57±0.35	90.73±0.22

(*Average value of 3 replicates)

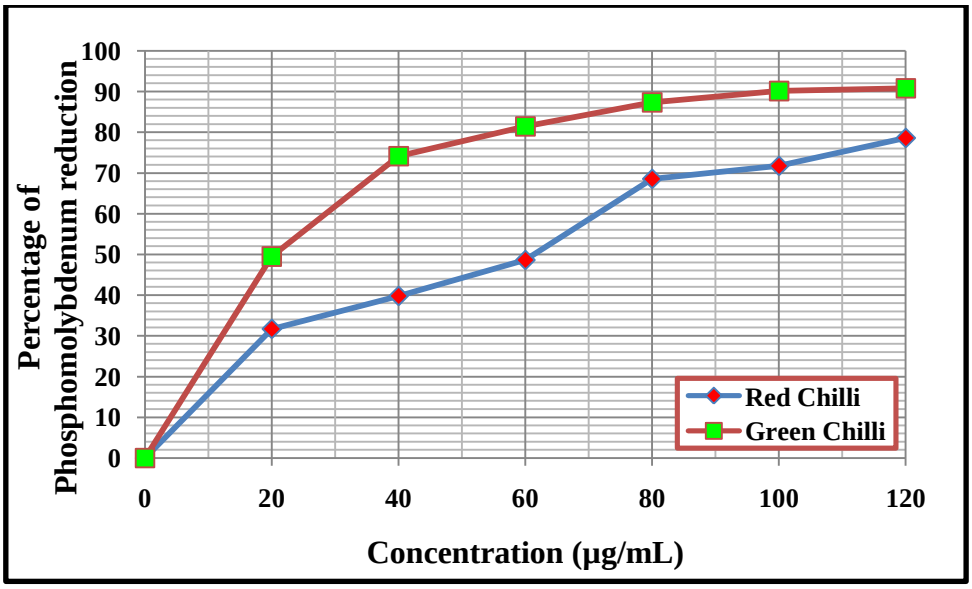


Figure 6. Phosphomolybdenum reduction activity of ethanol extracts of green and red chilli of *C. annuum.*

Ferric (Fe^{3+}) reducing power activity

The reducing power of Fe^{3+} to Fe^{2+} by the methanol extract of red and green fruit of *C.annuum* was studied and showed reduction ability in a dose dependent manner (Figure 3). The maximum reduction for ethanol extract of red chilli was 84.78% at 120µg/mL concentration. Comparatively, higher the ferric reducing potential for ethanol extract of green chilli was 88.42% at 120µg/mL concentration was observed (Figure 7). The RC_{50} value for ethanol extract of green chilli was found to be 41.54µg/mL concentration (Table 7) and was compared with the standard (29.11µg/mL concentration) Ascorbic acid.

Table 7. Ferric (Fe^{3+}) reduction activity of ethanol extracts of green and red chilli of *C. annuum.*

S.No	Concentration (µg/mL)	% of Fe^{3+} reduction*	
		Red Chilli	Green Chilli
1	20	33.33±0.33	31.80±0.22
2	40	63.15±0.42	48.14±0.38
3	60	75.62±0.10	80.79±0.41
4	80	80.97±0.43	81.06±0.29
5	100	83.56±0.27	83.57±0.15
6	120	84.78±0.18	88.42±0.36

(*Average value of 3 replicates)

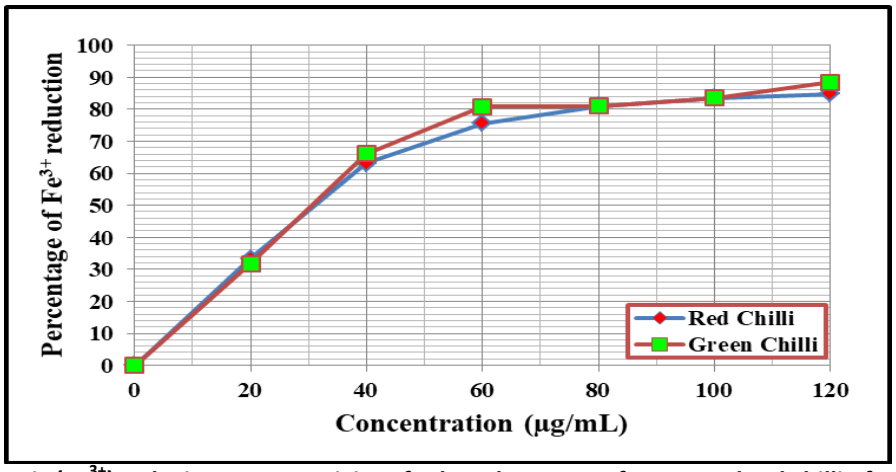


Figure 7. Ferric (Fe^{3+}) reducing power activity of ethanol extracts of green and red chilli of *C. annuum.*

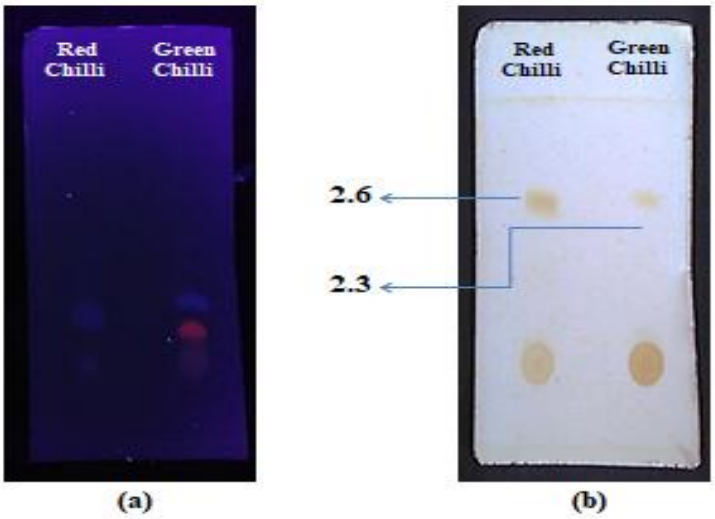


Figure 8. Compounds separation separated by Thin Layer Chromatography.
(a) Under UV light (b) Under Iodine stain

Thin Layer Chromatography analysis

Thin layer chromatography analysis was carried out in the solvent system of Toluene (2mL). The separated compounds in TLC were showed in Figure 8. The separated active compounds were visualized in UV light at 254nm and iodine balls. The R_f values of the separated compounds were measured and tabulated (Table 8).

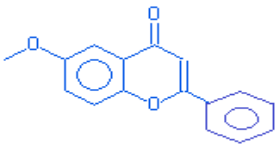
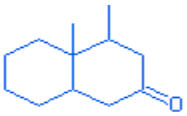
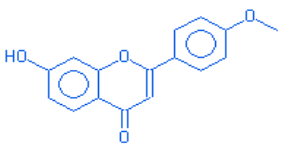
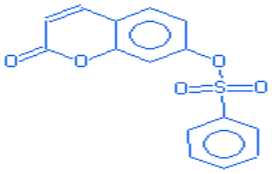
Table 8. R_f values of active compounds separated by Thin Layer Chromatography from the ethanol extract of Red Chilli and Green Chilli of *C. annuum.*

Number of spots	R_f value (Under Iodine)
Red Chilli – 1	2.6
Green Chilli – 1	2.3

GC-MS analysis

GC-MS analysis was carried out for the ethanol extract of Red Chilli and Green Chilliand the eluted compounds were showed in Table 9 and 10. Bioactive compounds such as Coumarine-7-phenyl sulphonyloxy, Piperidine-2-carboxamide, 1-amino-N-mesityl were identified from ethanol extract of Red Chilli. Comparatively, Phytol, Coumarine,3-[2-(1-methyl-2-imidazolylthio)-1-oxoethyl]- were identified from ethanol extract of Green Chilli. Presence of these compounds could be the reasons for the antioxidant property of the extracts.

Table 9. GC-MS analysis of ethanol extract of Red Chilli of *C. annuum.*

S.No	COMPOUND NAME	RT	COMPOUND STRUCTURE	MOLECULAR WEIGHT (g/mol)	MOLECULAR FORMULA
1	6-Methoxy flavone	17.78		252.26	$C_{16}H_{12}O_3$
2	2(1H)-Naphthalenone, octahydro-4,4a-dimethyl-(4a, 4aa,8aa)-	14.17		158.2	$C_{11}H_{10}O$
3	Dodecanoic acid, 10-methyl-methyl ester	17.07		214.34	$C_{13}H_{26}O_2$
4	4H-1-Benzopyran-4-one, 7-hydroxy-2-(4-methoxy phenyl)-	18.8		146.14	$C_9H_6O_2$
5	Coumarine-7-phenyl sulphonyloxy	19.63		302.3	$C_{15}H_{10}O_5S$

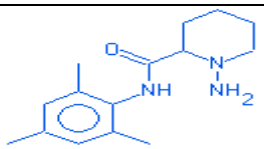
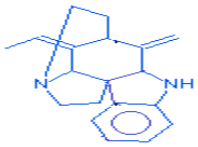
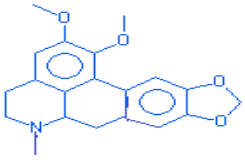
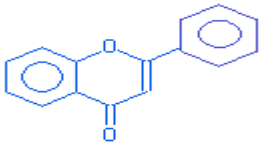
6	Piperidine-2-carboxamide,1-amino-N-mesityl	20.83		128.17	$C_6H_{12}N_2O$
7	Condyfolan, 14,19-didehydro-16-methylene-(14E)-	23.1		278.39	$C_{19}H_{22}N_2$
8	4H-Benzo[de][1,3] benzodioxolo[5,6-g]quinolone,5,6,6a,7-tetrahydro-1,2-dimethoxy-6-methyl-(s)-	25.77		454.48	$C_{27}H_{22}N_2O_5$

Table 10. GC-MS analysis of ethanol extract of Green Chilli of *C. annuum.*

S.No	COMPOUND NAME	RT	COMPOUND STRUCTURE	MOLECULAR WEIGHT (g/mol)	MOLECULAR FORMULA
1	Flavone	16.57		222.24	$C_{15}H_{10}O_2$
2	Hexadecanoic acid, methyl ester	17.07		270.45	$C_{17}H_{34}O_2$
3	n- Hexadecanoic acid	17.73		256.43	$C_{16}H_{32}O_2$
4	Phytol	18.8		296.53	$C_{20}H_{40}O$
5	Oleic acid	19.4		282.46	$C_{18}H_{34}O_2$
6	Nonadecanoic acid, 18-oxo, methyl ester	20.83		312.49	$C_{19}H_{36}O_3$
7	Coumarine,3-[2-(1-methyl-2-imidazolylthio)-1-oxoethyl]-	22.42		288.32	$C_{14}H_{12}N_2O_3S$

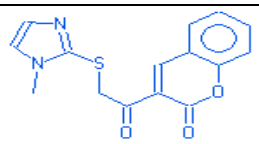
					
8	1,9,12-octadecatriene,1-methoxy-	23.1		278.48	$C_{19}H_{34}O$

Table 11. Bio-activity of ethanol extract of Red Chilli and Green Chilli of *C. annuum* from GCMS analysis.

S.No	COMPOUND NAME	PHARMACOLOGICAL ACTIVITY*
1	Coumarins	➢ Anti-inflammatory ➢ Anti-coagulant ➢ Anti-cancer ➢ Antimicrobial ➢ Alzheimer's disease inhibition ➢ Cytostatic effect
2	Piperidine	➢ Antioxidant ➢ Antimicrobial ➢ Anti-cancer
3	Phytol	➢ Aromatic Ingredient ➢ Antinociceptive ➢ Antioxidant ➢ Antiallergic ➢ Anti-inflammatory ➢ Antimicrobial ➢ Immunostimulant
4	Flavone	➢ Production of Reactive Oxygen Species (ROS) can be reduced by flavonoids. ➢ Relevance of plant defense mode of action is highly possible by flavonoids. ➢ Formation of oxygen radicals can be prevented by flavonoids thereby inhibiting the enzyme activity,
5	Oleic acid	➢ 5 alpha reductase inhibitor ➢ Hypocholestrolemic activity ➢ Perfumery and flavour ➢ Cancer preventing agent ➢ Anti-inflammatory activity ➢ Antibacterial activity

***(Divya Gupta et al. 2015; Syeda Nishat Fathima, 2015; Sergeant and May, 1970; Ryu et al. 2011; McGinty et al. 2010; Elaiyaraja and Chandramohan, 2016; Egan et al. 1990; Inoue et al. 2005; Lim et al. 2006; Kayser and Kolodziej, 1997; Justyna Mierziak et al. 2014; Jain and Himanshu Joshi, 2012)

CONCLUSION

Natural products discovered from medicinal plants have provided a great pathway for clinically approved medicines. The approval of the selected medicines falls under pharmacokinetics and pharmacodynamics (response of both drug and the human system). This is highly possible and can be proved by in-vivo animal model studies resulting in the approval of proper drugs.

Pharmacology, toxicology, dosage concentration and adverse effects produced by the medicines when consumed should be mainly targeted during clinical trials. Selection and screening the natural products is highly considerable since they are turned as NME's (New Molecule Entity) candidates for discovery and development of drugs. From the present investigation the fruits of ethanol extract of Red chilli and Green Chilli of *C. annuum*possessed significant antioxidant activity, due to the presence of phytochemicals. Comparatively, green chilli exhibited excellent antioxidant potential than red chilli. Further the active compound can be purified by column chromatography and crystallographic studies to evaluate the pharmacological activity of new active drug.

ACKNOWLEDGEMENT

The authors wish to thank Armats Biotek Training and Research Institute and SAIF, IIT, Madras for providing necessary facilities needed for the research.

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