

Comparative Analysis of Phytochemicals and Antioxidant Free Radicals Scavenging Activity of *Capsicum Annuum* L. and *Capsicum Frutescens* L.

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ABSTRACT

Many different types of chemicals found in medicinal plants have significant therapeutic qualities that can be applied to the treatment of human illnesses. Approximately 80% of people in rich countries use traditional medicine that is based on medicinal herbs. Usually fragrant and peppery, spices are plant-based goods. The presence of different kinds of essential oils in these plants has been proposed as the source of these characteristics. The naturally broken-down dried plant parts- fruits, seeds, bark, roots, and flowers – are specifically utilized to taste, season, colour and preserve food products. When compared to the distilled water extract, the metabolic extract of leaves showed the highest total phenolic content and the highest total flavonoid content, indicating a greater number of phytochemicals. High antioxidant capacity was also demonstrated by the leaves' methanol extract. Additionally, the potential purification of phytochemicals found in methanolic extract could aid in pinpointing specific phytochemicals, creating phytochemical profiles, and examining how they differ across the same species thriving in various habitats or *Capsicum* spp.

Keywords: Qualitative analysis, Quantitative analysis, Free radical scavenging activity

INTRODUCTION

The physiologically active substances found in many plant components, including leaves, flowers, seeds, bark, roots, and pulps, are known as phytochemicals. These are organic substances that have a clear and significant physiological effect on both humans and other animals. The phytochemicals are categorised into primary and secondary metabolites according to their physical, chemical, protective or curative properties, functional groups, and metabolic processes. Secondary metabolites are substances that plants make as byproducts of primary metabolite pathways and do not directly contribute to the growth, development, and continuing of the plants' offspring. abiotic stress, which is essential to the biosphere's ecology, competitiveness, and species interactions. These secondary metabolites contribute to the plant's colour, aroma, and flavour. These plant's phytochemicals are also essential for preventing illness and enhancing general health. The identification of these chemical compounds has prompted research into commercial and industrial

areas, including the fields of cosmetics, spas, painting, clothing, leather, traditional or alternative medicine systems, pharmaceuticals, dietary supplements, food, wine, and beverage industries, defence and military, etc. These developments have a significant impact on economy and social norms as times have changed. A new era of multidisciplinary areas using ethnobotany, in silico study methodologies, nanobiotechnology, molecular docking processes in bioinformatics, and cheminformatics has also been opened by the discovery of phytochemicals and functional groups and the recognition of existing ones. The interdependence of phytochemical structure and activity is crucial for the development of new substances and medications as well as the enhancement of numerous medicinal products. As a pepper variety they are annual plant or short – live *Capsicum annuum* L. can reach a height of 0.3 to 1.2 meters and *Capsicum frutescens* L. leaves are smooth and thin, 30-120cm long branches of plant. Current work has been conducted on the species *Capsicum annuum* L. and *Capsicum frutescens* (Linnaeus). Phytochemical screening and antioxidant activity of

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the selected plant have reported further and preliminary phytochemical screening also by (Hungund and Pathak in 1971). The purpose of current research on *Capsicum annum* L. and *Capsicum frutescens* L. is to perform preliminary phytochemical screening, quantitative phytochemical analysis, and check antioxidant potential, which might to be helpful to explore its use in the pharmaceutical industry and therapeutics.

MATERIALS ANF METHODS

2.1 Collection of Plant Material

Fresh leaves of two cultivated varieties of *Capsicum annum* L. and *Capsicum frutescens* L. were collected from the farm of Rampara of the Barwala district and their seeds to grow plant from shop of Panvi of (Shriji Agro) of Bhavnagar district. The collected leaves of the plant were washed carefully with distilled water, segregated and air-dried, then ground in the mixer grinder to produce a fine powder.

2.2 Plant Extraction

The cold extraction method is applied here, in which the dried, powdered material is macerated in the solvent on a shaker for 24 hours. Using analytical weighing balance and dissolved in methanol and distilled water in 1:20 ratio, respectively, in flasks. For 24 hours, the flasks were shaken. Next, the solutions prepared are filtered using Whatmann filter paper No.1. After filtration, the samples were poured into different petri plates and air- dried. For further procedures, the extract- containing Petri-plates are sealed with tape and stored in a laboratory refrigerator. The percentage yield of the six extracts is calculated using the following standard formula.

Percentage yield= Weight of the Dry Extract ÷ Weight of Plant powder taken) × 100

2.3 Qualitative Phytochemical Analysis

A stock solution of plant extract was prepared in order to conduct a qualitative phytochemical examination. The stock solution is made using a 1:1 plant extract to solvent ratio 30 ml of both plants' leaf extracts is dissolved in 30ml (1mg/ml) of methanol solvent. Labat test, alkaloids, carbohydrates, flavonoids, proteins, phenolics, tannins, saponin, terpenoids were

all screened for phytochemicals using established test protocols.

ALKALOIDS

Mayer's Test: 1ml of filtrate is treated with 2 ml of Mayer's reagent. The formation of a creamy white to yellow ppts/colouration indicates the presence of alkaloids.

Dragendoff's Test: 1 ml of filtrate is dissolved with a few drops of Dragendoff's reagent. The presence of alkaloids is confirmed by the appearance of a reddish-orange ppts/colouration.

CARBOHYDRATES

Molisch's Test: 1ml of Molisch's reagent is added to 1ml of filtrate. A positive test for the presence of carbohydrates is shown by the creation of a red or dull violet- coloured ring at the interface of two layers.

Benedict's Test: 2ml of Benedict's reagent is added to 2ml filtrate. Then let it boil for two minutes. Reducing sugars are indicated by the development of an orange- brick-red ppts.

Fehling's Test: 1ml of Fehling's A and equal amounts of Fehling's B solution are added to 1ml of filtrate. Then let it boil for two minutes. A positive test for carbohydrates is indicated by the formation of a brick-red precipitate.

PROTEINS

Million's Test: 1ml Million's reagent is added to 1ml of filtrate. The presence of proteins is demonstrated by the development of a white precipitates or colouration.

FLAVONOIDS

Lead Acetate Test: A few drops of 10% Lead Acetate solution are added to 1ml of filtrate. There are flavonoids present when a yellow precipitate forms.

H₂SO₄ Test: A few drops of concentrated Sulfuric Acid are added to 1ml of filtrate. The development of orange or red colouring indicates the presence of flavonoids.

Aluminium Chloride Test: Four to five drops of a 1% aluminium chloride solution are added to 2 millimeters of filtrate.

TANNINS:

FeCl₃ Test: A 1% lead acetate solution is added to 1 ml of filtrate. When a yellow-coloured ppt forms. The tannins test is affirmative.

Lead Acetate Test: A 1% lead Acetate solution is added to 1ml of filtrate. When a yellow- coloured ppt forms, the tannin test is affirmative.

SAPONINS:

Froth/Foam Test: 1ml of filtrate is dissolved in 2ml of distilled water, taken in a test tube and shaken vigorously' for 15-20 minutes. Let it stand for about 10-15 minutes. The persistence of foam for a few minutes is evidence of the presence of saponins.

TERPENOIDS:

Salkowski's Test: 1ml of filtrate is treated with 2ml of chloroform and 2ml concentrated H₂SO₄, which forms 2 layers. The formation of reddish-brown/rusty colouration at the interface depicts the presence of Terpenoids.

Copper Acetate Test: 2ml of filtrate is dissolved in distilled water and treated with 2ml of 5% Copper Acetate solution. The formation of emerald green colouration shows the presence of terpenoids.

PHENOLICS:

Brayer's Test (FeCl₃) Test: 1ml of filtrate is treated with a few ml of 5% FeCl₃ solution. The development of Bluish-Black to dark green colouration depicts a positive test for phenols.

Folin-Ciocalteu's Test: 1ml of filtrate and 1ml of FC reagent are mixed together. The formation of Bluish-green to a dark green colour or dark blue indicates the presence of phenolics compounds.

2.4 Quantitative Phytochemical Analysis

Determining the precise quantity of phytochemicals present requires a quantitative examination of their constituents. The stock solution was made with

concentration of 1mg/ml in 99% methanol solvent in order to perform quantitative analysis. The stock solution was made by dissolving 30mg of crude extract in 30ml of 99% methanol. Likewise, standard solutions of Gallic Acid (TPC) and Quercetin (TFC) and Tannic Acid (TTC) were made at a concentration of 1mg/ml.

2.5 Determination of Total Phenolic Content (TPC)

The Folin-Ciocalteu's assay process, which is a variation of the Folin- Denis's test procedure, was used to ascertain the total phenol content of the plant sample. After that 1ml of Folin-Ciocalteu's reagent is added, and 4ml of freshly made 20% Na₂CO₃ was added to the series; 30mL was added to dilute the series; and the allowed to sit in the dark for 30-40 minutes. Duplicates of the samples were measured using a spectrophotometer and readings of absorbance of the wavelengths as optical density (O.D.) at wavelength is 650nm. The total phenolic content of each sample was calculated in term of mg GAE/g of the sample (mg GAE/g = milligrams of gallic acid equivalent per gram of sample) the standard calibration graph of quercetin ($y = 0.4571x + 0.1205$; $R^2 = 0.9987$) was employed. Using the regression equation derived from the graph and the comparable process for stock solution for unknown samples (1mg/ml) using the following formula: $GAE = C \times V/M$ where C is the sample's concentration, expressed in mg/ml, determined by solving the standard calibration graph's equation.

Were,

C= concentration of sample obtained from solving the equation of standard calibration graph (mg/ml)

V= Volume of sample (ml) and

M= Mass of extract measured in grams.

2.6 Determination of Total Flavonoid Content (TFC)

0.1 ml of freshly made 10% AlCl₃ and 0.1ml (100μL) of 1M Potassium Acetate are added to 1ml of plant extract in order to estimate the total flavonoid content after that, 40 ml of distilled water were added to dilute the solution, and it was incubated for 30-40 min. A spectrophotometer set at 415nm was used to measure duplicates of the sample. To determine the total flavonoid concentration of an unknown sample, the

standard calibration graph of quercetin ($y = 0.0582x + 0.0332$; $R^2 = 0.9942$) was employed. The formula is used to express the flavonoid content in mg of quercetin equivalent per gram of sample, or mg QE/g of the sample), using the formula: $QE = C \times V/M$

Were,

C= Concentration of sample, expressed in mg/mL, that is determined by solving the equation of standard calibration graph.

V= Volume of sample used (mL) and

M= Mass of extract taken in grams.

2.7 Determination of Total Tannin Content (TTC)

The total tannin content of plant extract is estimated through the colorimetric assay. For estimating total tannin content, 0.5ml of Folin-phenol and 1ml (35%) of Na_2CO_3 are added to 1ml of plant extract. Then the solution was diluted with 10ml distilled water and incubated for 30 min. duplicates of the sample were measured using spectrophotometer at a 715nm wavelength. The standard calibration graph of tannic acid ($y = 0.3462 + 0.118x$; $R^2 = 0.9989$) was used to estimate the total tannin content of an unknown sample. The amount of tannin content is expressed in terms of mg TAE/g of the sample (milligrams of tannin equivalent per gram of sample), using the formula: $TAE = C \times V/M$

Were,

C= Concentration of sample obtained from solving the equation of standard calibration graph (mg/ml)

V= Volume of sample (ml) and

M= Mass of extract taken in grams.

2.8 Free Radical Scavenging activity-DPPH Assay

Using the DPPH assay, the plant sample was determined for their ability to scavenge free radicals. 4mg of DPPH solution was added to series of plant extract. Then the solution was allowed to sit at room temperature in dark for 20 to 30 minutes. At 517nm, the sample's absorbance was measured. Following formula was used for calculated DPPH scavenging Activity:

DPPH scavenging activity (%) =

$\left[\frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \right] \times 100$

2.9 Statistical Analysis

To reduce error, all quantitative phytochemical analysis results are performed in duplicate. The mean standard deviation (S.D.) is used to represent the average of these results. The Microsoft Office 2019 Excel program was used to do the statistical analysis computations. To further calculating the TPC, TFC, TTC, and DPPH assay, several regression equations for the straight line, $y = mx + c$, are derived from the four graphs. In these equations, y stands for absorbance of extract, m for graph slope, x for extract concentration, and c for graph intercept.

RESULTS AND DISCUSSION

Table-1: Percentage yield of *Capsicum annum* L. And *Capsicum frutescens* L. plant extracts.

Sr. no.	Plant Parts (leaves)	Methanol	D.W
1	<i>C. annum</i> L.	0.22%	0.18%
2	<i>C. frutescens</i> L.	0.21%	0.17%

Table-2: Qualitative Phytochemical Analysis of both Species

Test	Leaves <i>C. annum</i> (L.)	Leaves <i>C. annum</i> (L.)	Leaves <i>C. frutescens</i> (L.)	Leaves <i>C. frutescens</i> (L.)
	Methanol	D.W	Methanol	D.W
Alkaloids				
Mayer's Test	+	+	-	-
Dragendoff's Test	+	+	-	-
Carbohydrates				
Iodine Test	-	-	-	-
Fehling Test	+	+	+	+
Benedict's Test	+	-	+	-
Proteins				
Million's Test	+	+	+	+
Phenolics				

Brayer's Test	+	-	+	-
FeCl ₃ Test				
Lead Acetate Test	+	+	+	+
Folin-Ciocalteu's Test	+	-	+	-
Flavonoids				
Lead Acetate Test	+	+	+	+
H ₂ SO ₄ Test	+	-	+	-
Aluminium Chloride Test	+	-	+	-
Tannins				
FeCl ₃ Test	+	-	-	-
Lead Acetate Test	+	+	+	+
Saponin				
Froth Test	+	-	+	-
Terpenoids				
Salkowski's Test	+	+	-	-
Copper Acetate Test	+	-	-	-
Labat Test				
Gallic Acid+ Olive Green	+	+	+	+
Carboxylic Acid				
Na ₂ CO ₂ Test	+	-	+	-

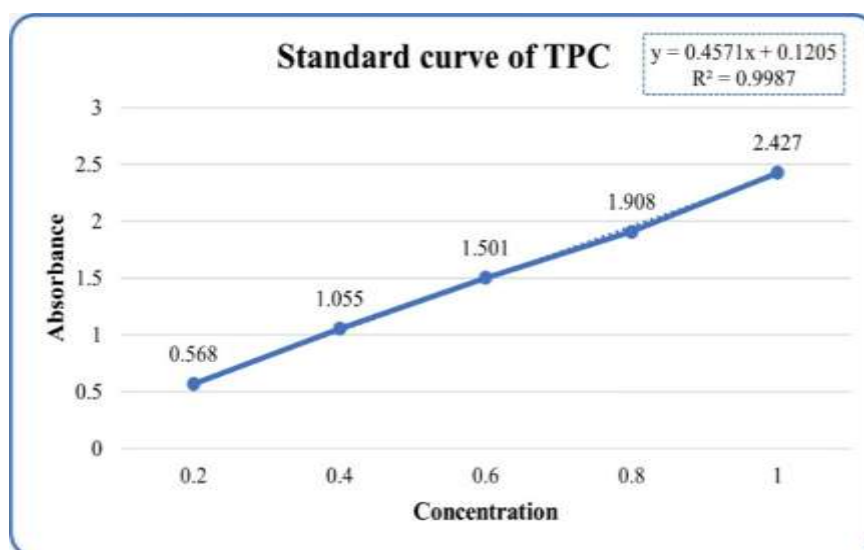
('+' Indicate presence of phytochemical, '-' indicate absence of phytochemical)

Table-3: Quantitative phytochemical analysis of both Species

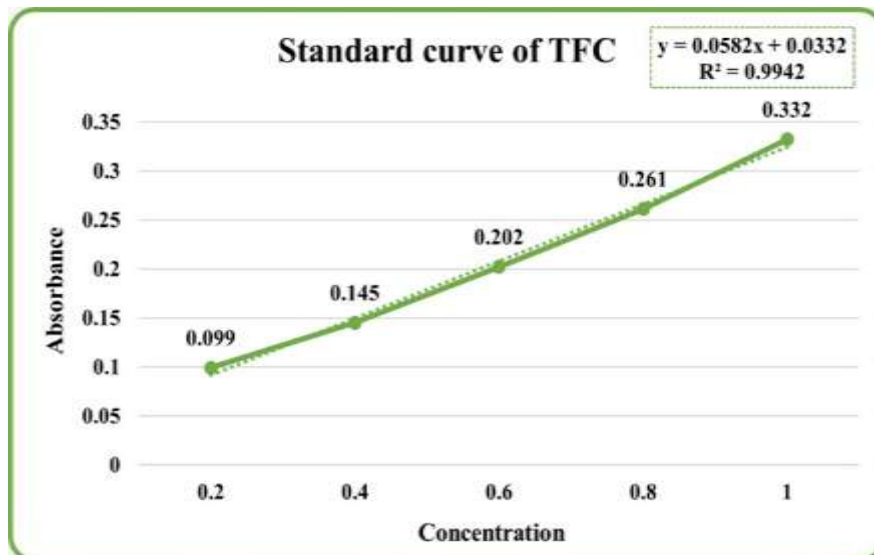
Sr.no	Content	Leaf		Leaf	
		Methanol	D.W.	Methanol	D.W.
1	TPC (mg GAE/g)	0.482	0.043	0.453	0.096
2	TFC (mg QE/g)	1.731	3.347	1.697	1.508
3	TTC (mg GAE/g)	0.450	0.670	0.678	0.589

Table- 4: Antioxidant activities of both the Species

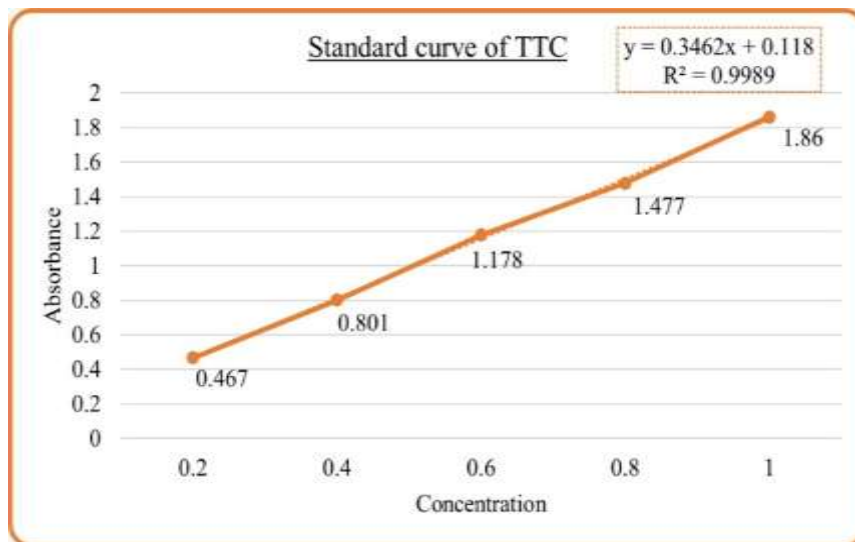
Sr. No.	Content	Leaf		leaf	
		Methanol	D.W.	Methanol	D.W.
1	DPPH	13.288±16.955	8.736±15.563	3.865±16.55	12.851±16.767



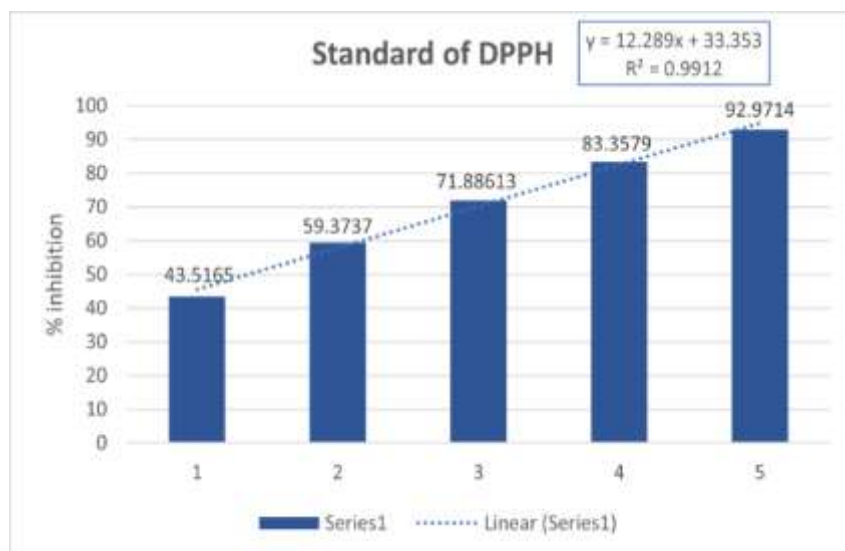
Graph-1: Standard Calibration Curve for Total Phenolic Content (TPC)



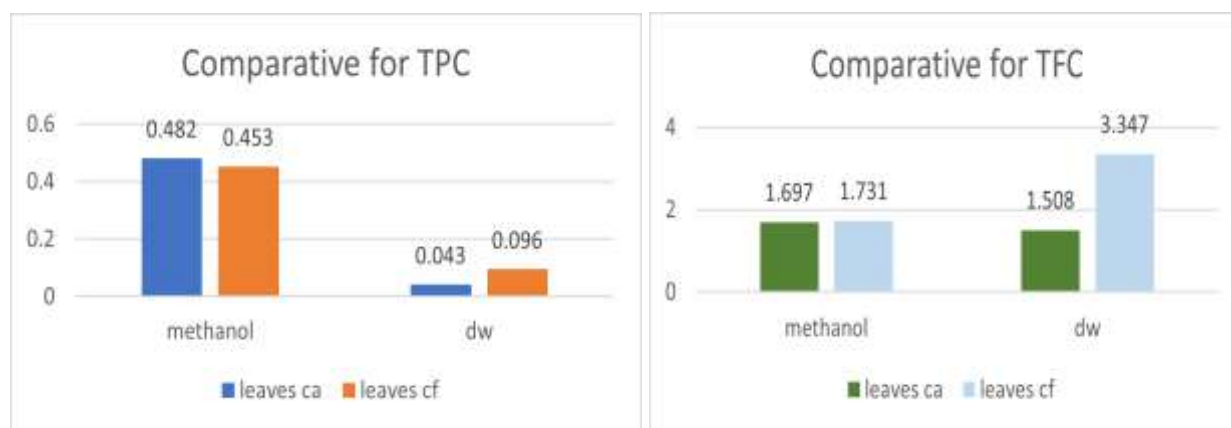
Graph-2: Standard Calibration Curve for Total Flavonoid Content (TFC)



Graph-3: Standard Calibration curve for Total Tannin Content (TTC)



Graph-4: Standard Calibration curve for Free Radical DPPH assay



Graphs 5,6: Showing the results of Comparative for TPC & TFC for Plant Samples



Graphs 7&8: Showing the results of Comparative for TTC & Free Radical (DPPH) for Plant Samples

DISCUSSION

The aim of this plant species study was to examine the different secondary metabolites of this plant, which can then be used as a raw material in a variety of industries. The methanolic extract of both plant leaves contained all of the important phytochemicals, such as, flavonoids, tannins, proteins, saponins, carbohydrates and had the maximum yield of dry extract, as shown in Table 1 and 2. Conversely, the plant leaves distilled water extract had the fewest phytochemicals. Quantitative analytical results showed that the methanolic extract of leaves had the highest levels of antioxidant activity, total phenolic content, and total flavonoid content (Table 3). The highest total phenolic content is found in the leaves of *Capsicum annuum* L. (0.482 ± 0.006 mg GAE/g of sample as compared to that of *Capsicum frutescens* L. 0.453 ± 0.009 mg GAE/g of sample (Rutuba *et al.*, 2021), 1.697 ± 0.015 mg QE/g of sample as compared to *Capsicum frutescens* L. 1.731 ± 0.006 mg QE/g of sample which shows flavonoid content as maximum amount (Patel & Modi, 2020). Whereas leaves of *Capsicum frutescens* L., methanol shows the highest

amount of total Flavonoid content with 1.731 ± 0.006 mg QE/g of the sample as compared to that of *Capsicum annuum* L. 1.697 ± 0.015 mg QE/g of sample. Leaves of *Capsicum frutescens* L. methanol shows the highest amount of total Tannin content with 0.678 ± 0.056 mg TAE/g of sample as compared to *Capsicum annuum* L. 0.450 ± 0.004 mg TAE/g of samples respectively. Antioxidant Activity was performed by DPPH assay in our study. In which Ascorbic acid was used as standard. In this study, the IC_{50} value methanolic plant extract and standard was 1.354 mg/ml and plant extract with 40.492 ± 25.139 mg/ml and 15.074 ± 37.569 mg/ml at $60 \mu\text{g/ml}$ concentration with 50% IC_{50} value, compared with control readings of methanol and distilled water of samples respectively. (Park J. H., *et al.*, 2012) investigated antioxidant activity using the DPPH Free Radical Scavenging Assay. The antioxidant components, stability, and function of *Capsicum annuum* L. and *Capsicum frutescens* L. leaves were investigated in vitro in this study. The DPPH free Radical Scavenging Assay has been used extensively to assess the ability of different natural products to scavenge free radicals that arise in lipids. Further

research is required to confirm its antioxidant qualities in dietary and biological systems. Their examination of the antioxidant activity revealed a negative correlation between the extract's antioxidant activity and its IC_{50} value, which is obtained by linearly regressing the DPPH scavenging activity against extract concentrations.

CONCLUSION

The plants being studied, *Capsicum annuum* L. and *Capsicum frutescens* L., are rich in flavonoids, total phenolics, total tannin and antioxidants. Wherein *Capsicum frutescens* possesses strong antioxidant, phenolic, and flavonoid qualities. The phytoconstituents must be further purified, especially the secondary metabolites including flavonoids, phenolics, alkaloids, and tannins, which were shown to be most prevalent in the methanolic extract of leaves utilizing a variety of chromatography techniques and contemporary methods. According to phytochemical screening, *Capsicum frutescens* L. has a larger flavonoids content than *Capsicum annuum* L. but a lower phenol level. For both methanol species and distilled water used as solvent, the protein content is minimum. Both distilled water and methanol did not contain alkaloids in *Capsicum annuum* L. species. Both the solvent methanol and distilled water are found in *Capsicum frutescens* L. This is due to the fact that phytochemical analysis of this plant has also gotten less attention. This could assist in determining the specific chemical structure of the phytoconstituents and developing a phytochemical profile of the plant. It can be used in a variety of basic and applied biology domains, including microbiology, biotechnology, nanotechnology, phylogeny and evolutionary biology, pharmaceuticals for drug creation, and bioinformatics for molecular docking, due to variations in ecological niches and resources constraints, the phytochemical profiles of the plants may be different from those of their counterparts growing in other environments.

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