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CONTRIBUTION TO THE QUALITY CONTROL AND *IN-VITRO* ANTIOXIDANT ACTIVITY OF LEAVES OF *PETROSELINUM CRISPUM* AND *CORIANDRUM SATIVUM* CULTIVATED IN SAUDI ARABIA

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Keywords:

P. crispum, *C. sativum*, Leaves, Physicochemical, TLC, Phenolic, Antioxidants

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ABSTRACT: The leaves of *Coriandrum sativum* commonly known as Cilantro and *Petroselinum crispum* commonly known as Parsley, family Apiaceae, have several health benefit effects. Most of the people, confused while buying these leaves. In the present study, comparative morphological, microscopic, fluorescence, TLC, and phytochemical parameters, comparative assessment of phenolic and flavonoid contents and *in-vitro* antioxidant activity of *C. sativum* and *P. crispum* leaves were carried out using standard methods. The leaves of these plants are morphologically similar, except other characteristics such as smells, number of lobes and serrate. The microscopic study showed the presence of different types of stomata, epidermis, fibers, and trichrome. Comparative fluorescence, ash content, LOD, and extractive content study were shown the distinct features. The total phenols and flavonoids of the leaves of *P. crispum* were higher than the leaves of *C. sativum*, and its antioxidant activity was also stronger than the leaves of *C. sativum*. The present comparative study reports the first time and can be useful for the identification or authentication of *P. crispum* and *C. sativum* leaves or its powder. The *in-vitro* study proved these edible green leaves may be useful for the dietary supplements.

INTRODUCTION: *Coriandrum sativum*, (Arabic-al-kuzbara) referred as a storehouse for bioactive compounds, belonging to the family Apiaceae, It is an Italian, inborn of Mediterranean regions, currently cultivated in Asia, and Central and Eastern Europe [1]. The seed of the *C. sativum* is called coriander and the herb leaf called Cilantro, is one of the warm climate annual plant.

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More than 28 days old *C. sativum* is commercially transported to the market for the customers when the leaves produced multiple petioles with variable leaf phenotypes [2]. *C. sativum* has many beneficiary effects such as upholding of health conditions, anti-oxidants, hypoglycemic, lowering of cholesterol, insecticidal, antibacterial, anti-mutagenic, mouth ulcer, antidiarrheal, anemia, smallpox, eye care and skin disorder [3]. Coriander oil is commonly used in baking foods, condiments, and curry. The phenolic compounds such as apigenin, luteolin, hesperidin, vicenin, diosmin, catechin, gallic acid, catechn, furelic acid, esculetin, dicoumarin, arbutin, catechin and *p*-coumaric acid, and aliphatic alkenals and alkanals were reported in *C. sativum* [4].

The most common form of Parsley is *P. crispum*, having a flat leaf, also native to the central Mediterranean region; it is used as a medicinal purpose before being eaten as a food [5]. *P. crispum* (Arabic-Al-bagdunis) is a biennial plant when the leaf stems have three segments; it is ready to be harvested. It has several varieties, but flat leaf Parsley is generally used in cooking because they have a better flavor in comparing to curly-leaf [6]. The extract of the leaves of this plant is a good source of flavonoids, flavonoid glycoside, furanocoumarin, Carotenoid, and ascorbic acid and traditionally used for various ailments [7, 8]. The leaves of *C. sativum* and *P. crispum* are available throughout the year and occupies a prime position in the condiment or flavoring substances. *C. sativum* and *P. crispum* both leaves are most confusing while purchasing, but most of the cases they are identified by smell, *C. sativum* leaves have a strong and characteristic smell in comparison to *P. crispum* leaves.

Almost all parts of both plants are comestibles; nevertheless, its fresh leaves and the dried seeds are frequently utilized. These green leaves, contains proteins, vitamins, minerals, fibers, and carbohydrates, is used as a vegetable, and in salads, soups, as it lessens the need for salt, while both the plant leaves contain essential oils, provide typical different flavor, when added to the food products and these acts as preservative [9, 10, 11]. The study of the literature revealed nonexistence of comparative analytical studies on the leaves of *C. sativum* and *P. crispum*. Hence, the present investigation was commenced with the objective to evaluate the comparative qualitative, and quantitative analysis of the *C. sativum* and *P. crispum* leaves, and comparative total phenolic and flavonoids content along with the *in-vitro* study of antioxidant of its methanol extract were carried out.

MATERIALS AND METHODS:

Chemicals and Reagents: All chemicals used in the study were of analytical grade. Folin-Ciocalteu reagent (FCR), gallic acid, quercetin, DPPH, and Ascorbic acid were purchased from Sigma-Aldrich Co. (St Louis, MO, USA).

Plants Collection and Processing: The leaves, *C. sativum*, and *P. crispum*, were purchased from the local market of Al-Kharj, Kingdom of Saudi Arabia in January 2017 and the plant species were authenticated by Dr. Osman A. O. Elmakki using the morphological features of the two plant samples. The voucher specimens were deposited at the herbarium, College of Pharmacy (PSAU-CPH 11-2017) and (PSAU-CPH 12-2017) consecutively, at Prince Sattam Bin Abdulaziz University, Al-Kharj, KSA.

The leaves were shed dried and powdered using a mechanical powder grinder. The grounded powder was stored in an airtight bottle for further study. About, 10 g of powder in methanol (200 ml) was sonicated for 1 h at room temperature, filtrate (Whatman 2) and the filtrates were removed under reduced pressure and it was further lyophilized. The fine powder was used for TLC fingerprinting, phytochemical study, estimation of phenolic and flavonoids contents, and *in-vitro* antioxidant activity.

Morphological, Microscopic, and Fluorescence Study: The photograph, free online tool converters (http://www.snapstouch.com/Sketch.aspx) of the fresh leaves of *C. sativum* and *P. crispum* was converted; the comparative observation of morphological dissimilarity between these leaves was recorded. The comparative microscopic study of leaves was carried out by using chloral hydrate reagents and phloroglucinol-HCl (1:1) reagent, Olympus America Inc., (Model no. BX 41) microscope with the analog camera (ProgRess C3-Jenoptik) was used for microphotography of the mounted slides. Computer images at × 10, × 20, and × 40 magnifications were captured. The fluorescence analysis of powder was carried out using the previous method [12].

Physicochemical Study of Powder: Determination of ash values (Total, acid insoluble and water soluble ash), the percentage loss on drying (% LOD) and extractive values were carried out using the reported method with slight modification [13]. Accurately, one gram of the powder was ignited using a furnace at 600 ℃ for 6 h, cooled at room temperature (25 ℃), total (mg/g), acid insoluble and water soluble ash were calculated. Percentage loss on drying (% LOD) was determined by placing accurately 5 g of powder in a china dish and it was dried in an oven at 105°. The percentage of extractive values were recorded in hexane, ethyl

acetate, and methanol. Accurately 10.0 g of powdered was placed in a conical flask containing glass-stoppered and sonicate (Sonicator®) with 200 ml of the solvent for 1 h. The mixture was filtered and it was transferred to a pre-weighted tared flat-bottomed dish and evaporated to dryness on a water bath.

TLC and Preliminary Phytochemical Study of Methanol Extract: The methanol extract was subjected to TLC (Thin Layer Chromatography) using the ascending method on silica gel 60F254, 10×5 cm (Merck) glass plates. The spots were made using glass capillaries. The twin trough chamber was saturated with ethyl acetate: methanol: water (E: M: W, 8:2:1 v/v/v) and toluene: ethyl acetate (92:8 v/v) for 20 min. The spotted plates were run, and the developed plates were dried and compare under UV 254 and 312 nm. Retention factor (R_f) was calculated by dividing the movement of spots with a distance of solvent front Secondary metabolites in the powder of *C. sativum*, and *P. crispum* leaves were detected by performing color tests [14]. Active constituents such as alkaloids, glycosides, flavonoids, tannins, and saponins were detected.

The Quantitative Study of Total Phenols and Flavonoids: Total phenolic and flavonoids content in the methanol extract of *C. sativum* and *P. crispum* leaves were quantified using UV/VIS spectrophotometer [15]. One gram of extract was sonicated three times (30 min each) with 15 ml of 50% methanol, and all filtrates were collected together and makeup to 100 ml with the similar solvent in a volumetric flask (10 mg/ml). Standard solution of total phenolic or flavonoids contents were prepared by weighing accurately, 20 mg of gallic acid and quercetin separately and diluted up to 100 ml in a volumetric flask using 50% methanol (200 µg/ml).

Further, serial dilution (5, 10, 20, 40, 80,100 and 200 µg/ml) of each standard was prepared for the quantitative analysis. For the total phenolic, the absorbance was taken at 765 nm, and the phenolic content was expressed as GAE in milligram per gram dry extract, and for the total flavonoid, absorbance was taken at 415 nm, and flavonoid content was expressed as quercetin in milligram per gram dry extract.

Antioxidant Activity: The assay of total antioxidant is based on the method of Ahmed *et al*. [16] Extract, (0.3 ml) was mixed with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solutions were incubated at 95 °C for 90 min. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer. The reducing assay was based on the developed method of Foudah [17]. A stock solution of standard (Ascorbic acid) and samples (1000 µg/ml) were prepared. The serial dilutions (10, 25, 50, 75 and 100 µg/ml) of each standard and samples were prepared, and the absorbance was noted at 700 nm in a UV spectrometer. The DPPH radical scavenging assay was based on the developed method [17]. The free radicals of DPPH showed a robust absorption at 517 nm. A serial dilution (10-1000 µg/ml) of ascorbic acid and methanol extract of each, *C. sativum* and *P. crispum* leaves were prepared.

Statistical Analysis: Values were expressed as average and mean ± SD of three measurements. The IC_{50} values were calculated by linear regression analysis.

RESULTS: The experiment was carried out to the leaves, powder, and methanol extract.

Morphological, Microscopic, and Fluorescence Analysis: FIG. 1, showed, a typical morphology of the *C. sativum* and *P. crispum* fresh leaves, both had similar shape and size, but some of the features are different. **Fig. 1a**- Both leaves are alternate, three-lobed with each lobe again three- to four-lobed but the tops of the *C. sativum* had seen teeth and rounded, but *P. crispum* had lobed and pointed. **Fig. 1b**- Number of leaflets are more in *C. sativum* than *P. crispum*, **Fig. 1c**- Leaf midrib of *P. crispum* is more prominent than *C. sativum*, **Fig. 1d**- Leaf blade divided into 3 leaflets, in both plants, but comparatively, *C. sativum* toothed had more in number (20-25) than *P. crispum* (15-20) leaf.

Fig. 2, showed, comparative powder microscopic differences between *P. crispum* and *C. sativum* leaves. **Fig. 2A**- showed, epidermis in oblique surface view, and different shapes were observed. **Fig. 2B**, showed lower epidermis, clearly distinguished from each other. **Fig. 2C**- Lower

epidermis of surface view, *P. crispum* show anomocytic stomata, while Coriander showed anisocytic stomata. **Fig. 2D**- different types of trichrome, *P. crispum* show unicellular covering trichrome while coriander show multicellular is covering trichrome. **Fig. 2E**- showed a different thickness of the fibers and **Fig. 2F**- shows fibro-vascular tissues. **Table 1**, showed fluorescence analysis of leaf powder under daylight and UV (Short, 254 nm and long, 312) lights. The chemical such as 1N NaOH (aqueous), acetic acid, H_2SO_4 (Conc.), HCl (conc.), KOH solution (aqueous), ammonia solution, $AgNO_3$ solution, distilled water, and hexane was used for comparative fluorescence analysis.

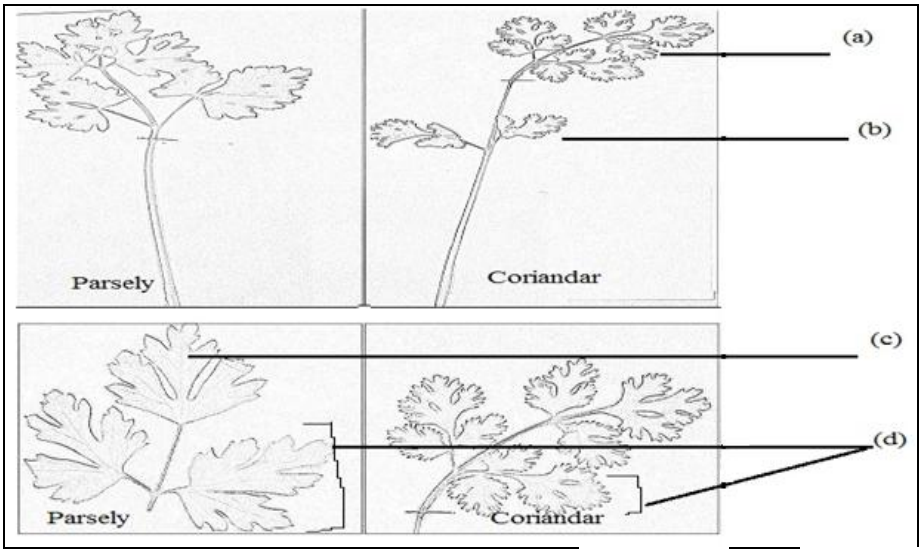

FIG. 1: MORPHOLOGICAL STUDY OF LEAF OF *P. CRISPUM* AND *C. SATIVUM*

FIG. 2: POWDER CHARACTERISTICS OF *P. CRISPUM* AND *C. SATIVUM* LEAF

TABLE 1: FLUORESCENCE ANALYSIS OF *P. CRISPUM* LEAVES AND *C. SATIVUM*

Reagents or solvents	Normal Light		Short UV (254nm) Light		Long UV (312nm) Light	
	P. crispum	*C. sativum*	*P. crispum*	*C. sativum*	*P. crispum*	*C. sativum*
1N NaOH (Aqueous)	NC	NC	White	Green	Brown	White
Acetic acid	NC	NC	Pink	Orange	Pink	Orange
H_2SO_4 (Conc.)	NC	NC	Gray	Cream	Gray	Cream
HCl (Conc.)	NC	NC	Light green	Brown	Light green	Dark Brown
KOH solution (Aqueous)	NC	NC	Brown	White	Orange	Silver
Ammonia solution	NC	NC	Silver	Cream	Silver	Cream
$AgNO_3$ solution	NC	NC	Cream	Brown	Cream	Brown
Dist. water	Brick red	Green	NC	NC	NC	NC
Hexane	NC	NC	Pink	Green	Pink	Orange/Red

Physicochemical Study of Powder: Table 2, showed the mean value of the comparative analysis of physicochemical parameters.

TABLE 2: PHYSICOCHEMICAL ANALYSIS OF *P. CRISPUM* LEAVES AND *C. SATIVUM*

Analysis	*P. crispum*	*C. sativum*
% Ash (Total)	7.42	7.52
% Ash (Acid insoluble)	1.72	1.85
% Ash (Water soluble)	4.32	4.45
% Loss on drying (LOD)	7.74	10.45
Hexane extract	2.4	2.48
Ethyl acetate extract	2.8	3.12
Methanol extract	19.28	12.96

*Values were average of triplet

The values of percentage total ash content, acid insoluble and water soluble ash content of powder of *P. crispum* and *C. sativum* leaves were (7.42, 1.72 and 4.32%) and (7.52, 1.85 and 4.4%) respectively. The LOD values, 7.74%, and 10.45% were shown for *P. crispum* and *C. sativum* leaves powder respectively. Extractive values such as hexane, ethyl acetate, and methanol for *P. crispum* was 2.4, 2.8. 19.8%, respectively, and for *C. sativum* was 2.48, 3.12 and 12.96%, respectively, and were different from each other.

TLC and Preliminary Phytochemical Study of Methanol Extract: Fig. 3, showed the comparative TLC spots, the mobile phase E: M: W (8:2:1) developed plate was showing a clear, distinct spot (R_f = 0.72) for *C. sativum* **Table 3**, at 254 nm, *P. crispum* showed no clear spot while *C. sativum* showed a clear spot. **Fig. 3A**, at 312 nm, with mobile phase E: M: W (8:2:1) both extracts showed distinct colour spots (RF: 0.74), the spots of *P. crispum* had ice blue in colour while *C. sativum* had violet in colour. **Fig. 3B**: at 254 nm, with a solvent system T: E (92:8), showed no clear, distinct spots, but at 312 nm both showed distinct colour spots (R_f: 0.21), *P. crispum* had a white colour spot, while *C. sativum* had brown colour spot. **Table 4**, showed the comparative phyto-chemical analysis of the powder of the leaves of *P. crispum* and *C. sativum* and the results were shown the presence of alkaloids, tannins, glycosides, flavonoids, and saponins.

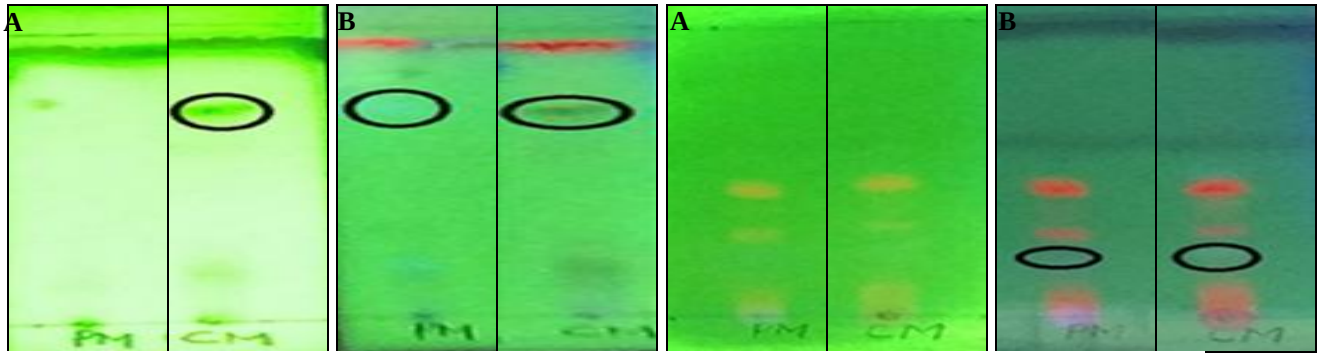

FIG. 3: TLC ANALYSIS OF THE METHANOL EXTRACT OF LEAF POWDER OF *P. CRISPUM* AND *C. SATIVUM*

TABLE 3: TLC ANALYSIS OF THE METHANOL EXTRACT FROM *P. CRISPUM* LEAVES AND *C. SATIVUM*

Samples	Observation	*R_f value, E:M:W (8:2:1)	R_f value, Solvent system T:E (97:3)
P. crispum	254 nm	NO	No
	312 nm	0.74 (Ice blue)	0.21 (White)
C. sativum	254 nm	0.74	No
	312 nm	O.74 (Violate)	O.21 (Brown)

*R_f =average of triplet, Where, E (Ethyl acetate), M (Methanol) and W (Distilled Water), T (Toluene)

TABLE 4: PHYTOCHEMICAL ANALYSIS OF *P. CRISPUM* LEAVES AND *C. SATIVUM*

Phytochemicals	Reagents	*P. crispum*	*C. sativum*
Alkaloids	Mayer's, Picric acid	+	+
Tannins	Ferric chloride	+++	++
Flavonoids	Lead acetate	+++	++
Glycoside	Br water,, Borntrager's	++	+
Saponin	Foam test	++	+++

Plentiful present: +++; Medium present: ++; present: +

Quantitative Study of Total Phenols and Flavonoids: The total phenol and flavonoid contents of the methanol extract of *P. crispum* and *C. sativum* were shown in **Table 5**. The total phenol content of methanol extract of *P. crispum* (114 mg GAE/g dry extract) was higher than *C. sativum* (108 mg GAE/g dry extract). Similarly, the total flavonoid content of methanol extract of *P. crispum* leaves (14.3 mg QE/g ME) was higher than *C. sativum* leaves (11.2 mg QE/g ME). The total phenolic content, was calculated using a calibration curve prepared by gallic acid (Y = 0.0142 X + 0.0896; R^2 = 0.9915) **Fig. 4A** and the flavonoid content was calculated using a calibration curve prepared with quercetin (Y = 0.0035 X + 0.0429; R^2 = 0.9959) **Fig. 4B**.

TABLE 5: PHENOLIC AND FLAVONOIDS CONTENTS, AND *IN-VITRO* ANTIOXIDANT ACTIVITY OF METHANOL EXTRACT OF *P. CRISPUM* LEAVES AND *C. SATIVUM*

Evaluation	*P. crispum*	*C. sativum*	Ascorbic acid
Total Phenolic* (mg GAE/g, dw)	114 ± 0.01	108 ± 0.11	-
Total Flavonoid* (mg QE/g, dw)	14.3 ± 0.21	11.2 ± 0.16	-
Total Antioxidant* Capacity (mg AAE/g)	107.2 ± 0.21	83.5 ± 0.05	98.6 ± 80.16
Reducing power* (Absorbance) at 100 µg/mL	0.231 ± 0.17	0.193 ± 0.09	0.442 ± 0.23
% DPPH** antioxidant assay at 1000 µg/mL	87.30	51.86	98.68
IC50** (µg/ml) (DPPH)	153.66	851.00	56.55

*Values were mean ± SD of triplet, **Average of triplet

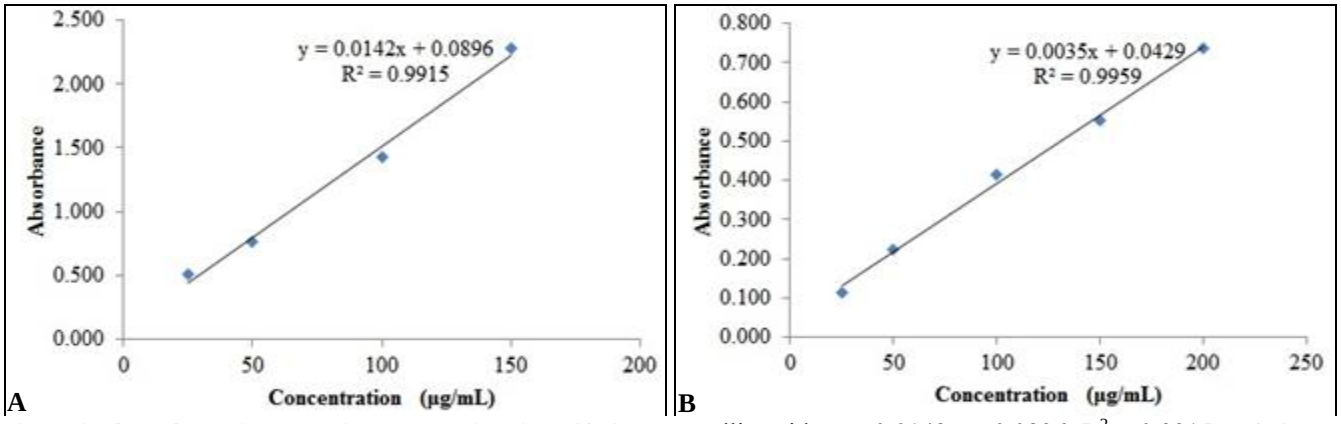

FIG. 4: STANDARD CURVE OF EXTINCTION. A) Against gallic acid "y = 0.0142x + 0.0896; R^2 = 0.9915". B) Against quercetin "y = 0.0035x + 0.0429; R^2 = 0.9959".

Antioxidant Activity: The **t**otal antioxidant capacity, reducing power, and DPPH radical scavenging action of the methanolic extract of *P. crispum* and *C. sativum* were shown in **Table 5**. The antioxidant activity of *P. crispum* (107.2 ± 0.21 mg AAE/g) was higher than *C. sativum* (83.5 ± 0.05 mg AAE/g).Reducing the power of both the samples was increased with the concentrations. The reducing power of *P. crispum* (0.231 ± 0.17) was higher than *C. sativum*, (0.193 ± 0.09) but, lower than ascorbic acid (0.442 ± 0.23) at 100 µg/ml. The results of DPPH radical-scavenging action of methanol extract of *P. crispum* and *C. sativum* were shown in **Fig. 5**.

The methanol extract of *P. crispum* had the highest percentage inhibition of DPPH (87.30%) than, *C. sativum* (51.86%) but lower than standard (98.68%) at 1000µg/ml. The IC50 value of *P. crispum* leaf extract (153.66 µg/ml) showed lower than *C. sativum* leaf extract (851 µg/ml) but higher than ascorbic acid (56.55 µg/ml).

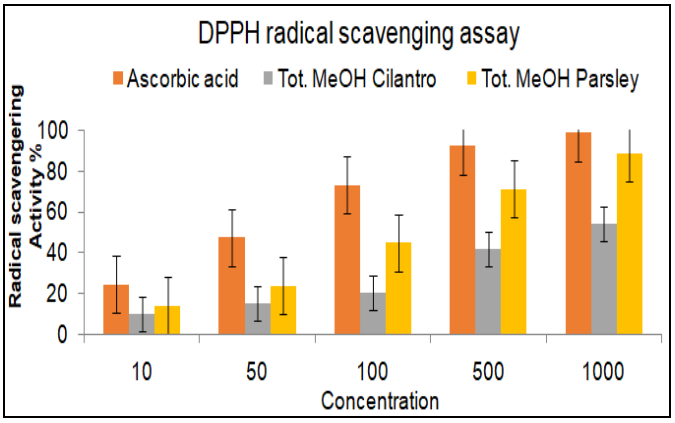

FIG. 5: PERCENTAGE DPPH FREE RADICAL SCAVENGING STUDY OF THE METHANOL EXTRACT OF LEAF POWDER OF *P. CRISPUM* AND *C. SATIVUM*

DISCUSSION: Morphological resemblances among some plant species and a lack of standard identification, contributing to accidental and deliberate substitution. The substitution and adulteration of plant parts in herbal products can cause health and safety concerns [18]. In present observation, the morphological structures of *P. crispum* and *C. sativum* leaves were showing that the different leaf margin, leaflets, and midrib are different from each other **Fig. 1**.

The microscopic analysis was shown several identification characters like epidermis, stomata, fibers, trichrome *etc*. **Fig. 2**. A fluorescence study is an important tool for the determination of chemical constituents and it gives an idea about the chemical nature of powder drugs [19]. The microscopic and fluorescence examination is one of the inexpensive methods for the correct identification of the plant powder. The comparative study of physiological parameters such as total ash, acid insoluble ash, and water soluble ash content of both the plants were different from each other and the results were illustrated in **Table 2**, a very small amount of the inorganic element is insoluble in acid and the present study showed that adulteration in the raw ingredients by other substances, is very low. The low acid-insoluble ash content affects the gastrointestinal absorption while is taken orally [20].

The loss on drying (LOD) content of powder drug is directly associated with its stability, the lower value of the LOD, the lower the chances of microbial growth and vice versa. The shelf life of the powder drug is inversely proportional to the moisture contents. The significant amount of LOD was found in both the leaf powder, so, the dried powdered of *P. crispum* and *C. sativum* should be stored in a dried form. The extracted value was calculated for hexane, ethyl acetate and methanol for both dried powders of *P. crispum* and *C. sativum* leaves and due to high polarity of methanol solvent, it was shown that the methanol extract recorded higher percentage yield than the other solvents.

The comparative study of the Thin Layer Chromatography (TLC) of the methanol extract of *P. crispum* and *C. sativum* leaves shows unique identification characters at low 254 nm and high 312 nm UV light **Fig. 3**. These characters may be helpful in the quality control of these plants. TLC method was commonly used for the herbal analysis and recently the other methods such as GC and HPLC methods were established, but still, it is frequently used for the detection of constituents, impurities, and active substances and it is commonly recommended by various pharmacopoeias [21]. The preliminary phytochemical analysis study of the *P. crispum* and *C. sativum* showed that the presence of active constituents such as alkaloids, tannins, flavonoids, glycosides, and saponins **Table 4**. These secondary metabolites are reported to have several biological and pharmacological properties, so this species is expected to use in various chronic diseases such as skin diseases, cancer cardio-vascular, and gastrointestinal disorders, *etc.* [22]

The secondary metabolites such as phenolics and flavonoids are the common antioxidants present in the medicinal plants, used for the prevention of chronic disease [23]. The current work was also carried out to compare the phenolics and flavonoid content in the methanol extract of *P. crispum* and *C. sativum* leaves. The study showed that more phenolic content was found in leaves of *P. crispum* than *C. sativum* **Table 5**. The previous study also reported that *P. crispum* leaf is a rich source of phenolic and flavonoids compounds [24]. Similarly, *C. sativum* is also known to be a rich source of phenolic and flavonoid compounds [25]. An imbalance between pro-oxidants and antioxidants in the human body caused oxidative stress (OS). Normal pro-oxidant is reactive oxygen species (ROS) and reactive nitrogen species (RNS) are free radicals in aerobic metabolism [26]. Total antioxidant capacity (TAC) of the methanol extracts of the

leaves of both species was evaluated using phosphomolybdate assay, where Mo^{6+} is reduced to Mo^{5+} in the presence of a reducing agent (antioxidant), forming a green Mo^{5+} complex [16].

Many natural products, including phenols and flavonoids, can cause this reduction. *P. crispum* leaves methanol extract showed a higher TAC than *C. sativum* methanol extract. The reducing power (RP) assay is frequently used to estimate the ability of antioxidants to donate an electron. The conversion of the green color of the ferric cyanide complex (Fe^{3+}) to the blue color of the ferrous cyanide form (Fe^{2+}) was observed in the presence of antioxidant compounds. The present study showed the high reducing activities of methanol extract of the *P. crispum* than *C. sativum* extract. In the present finding, DPPH scavenging activity was significantly higher in *P. crispum* leaves methanol extract than *C. sativum* methanol extract, in the dose-dependent mode and the mechanism is probably because of the more hydrogen-donating ability of *P. crispum* extract than *C. sativum* **Fig. 5**.

The higher DPPH scavenging may be due to the presence of the highest phenolic content of *P. crispum* leaves [27]. Kuzma *et al.*, [28] reported that *P. crispum* leaves have essential biologically active compounds such as vitamin C, carotenoids and chlorophyll. The antioxidant activity may be the result of the synergistic action of all the additional components, including phenolic and flavonoids contents, rather than of a single entity of the extract. The extracts of leaves may well act as donors of electron, and it can react with free radicals, terminate radical chain reactions by converting them into more stable products. Harsha and Anilakumar [29] reported that the ethanolic extract of *C. sativum* showed DPPH· scavenging activity, with IC_{50} values is comparatively more than that of the standard.

CONCLUSION: The current study might be useful to control the quality of *P. crispum* and *C. sativum* leaves. The *in-vitro* antioxidant studies showed that the methanolic extract of the leaves of both species possesses a significant level of total phenolic and flavonoids, and potent antioxidant activity measured in terms of TAC, reducing activity and DPPH scavenging assay. The standardization parameters which, are being reported first time in this work, could be useful in the preparation of the herbal monograph for proper identification and authentication of *P. crispum* and *C. sativum* leaves. The results of *in-vitro* studies were further justified that, leaves of *P. crispum* and *C. sativum* may be used as an alternative antioxidant for the food products and medicinal purposes.

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CONFLICT OF INTEREST: There is no conflict of interest.

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