



Appraisal of Antioxidant Profile of Two Underutilized Green Leafy Vegetables: Kasuri Methi and Chaulai

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ABSTRACT: Due to India's diverse environment, a large array of green leafy vegetables can be found, many of which are currently neglected. These vegetables require low-cost plantation and harvesting in addition to being tough and adaptable. Consumption of these vegetables that contain natural antioxidants is regarded to be an effective means of reducing the risk of oxidative stress diseases as well as metabolic disorders, since demand for sufficient food and nutrition has led to the scouting of more of these plants. The goal of this study was to determine the antioxidant content of selected underutilized green leafy vegetables, such as total phenols, total flavonoids, ascorbic acid, and β Carotene, as well as their antioxidant activity, such as FRAP and DPPH scavenging activity, using standard methods with minor modifications. The data of present study revealed that total phenols content ($301.61 \pm 1.19 \text{ mgGAE}/100\text{g}$) and total flavonoids ($285.40 \pm 0.01 \text{ mgQE}/100\text{g}$) were insignificantly higher in aqueous extract of Chaulai leaf whereas ascorbic acid ($22.13 \pm 0.18 \text{ mg}/100\text{g}$) and β carotene ($12.10 \pm 0.06 \text{ mg}/100\text{g}$) and FRAP $694.57 \text{ mmol}/100\text{g}$ were discovered to be substantially greater in Kasuri methi aqueous extract at $P < 0.05$ level. The data also indicated that Kasuri methi aqueous extract exhibits considerably higher DPPH scavenging activity with IC_{50} value ($15.3 \mu\text{g}/\text{ml}$) whereas Chaulai extract showed low scavenging activity $26.04 \mu\text{g}/\text{ml}$ when compared to control ($12.6 \mu\text{g}/\text{ml}$). As a result of this study, it shows that both underutilized leafy vegetables (Kasuri methi and Chaulai) can be used as a potential ingredient in the development of functional foods, and that their use could be a viable alternative to treating many chronic metabolic diseases.

Index Terms-Antioxidant, oxidative stress, phenols flavonoids FRAP, DPPH

I. INTRODUCTION

Various exogenic substances and endogenic metabolic activities produce free radicals, or highly reactive oxygen species in the human body. These are capable of oxidizing biomolecules and can cause neurological problems such as emphysema, cirrhosis, atherosclerosis, arthritis, and other degenerative diseases (Rizwan ahmad, 2018). Antioxidants are the substances that stop free radicals from attacking cells, lowering the risk of metabolic and degenerative diseases. Free radical damage is fought by enzymes like superoxide dismutase and catalase, as well as antioxidant substances such as ascorbic acid, tocopherol, phenolic acids, polyphenols, flavonoids, and glutathione. Natural antioxidants are currently receiving a lot of interest as a way to protect the human body, particularly brain regions, from oxidative damage caused by free radicals (Meena *et al.*, 2012). Antioxidant capabilities in plants are a prominent topic of study, particularly for lesser-known and underutilized plants and vegetables. Plant phenolic compounds have antioxidant properties that help protect cells from oxidative damage

produced by free radicals. One of the furthestmost significant recommendations for minimizing the risk of several diseases caused by elevated levels of free radicals is to eat a diet rich in vegetables (Akin-Idowu *et al.*, 2017).

Kasuri methi (*Trigonella corniculata*) belongs to a family *Fabaceae* and is used as a green vegetable (pot herb) especially in Northern parts of India. Its origin of cultivation is Kasur (a district of Punjab province), Pakistan and is a geographical indicator of that region (Erum *et al.*, 2011). It is used as flavouring agent and also known as protective food which reflects its significance in supplying of vital nutrients for good health (Singh *et al.*, 2019). It is known for its functional and nutraceutical properties such as-antibacterial, anticancer, antiulcer, hypocholesterolemic, hypoglycaemic, antioxidants and antidiabetic agent (Meghwal and Goswami, 2012).

Chaulai (*Amaranthus cruentus*) is a fast-growing plant that belongs to the *Amaranthaceae* family and is found all over the world. β -carotene, vitamins B6 and C, riboflavin, and folate, as well as vital minerals including calcium, iron, magnesium, phosphorus, potassium, zinc, copper, and manganese, are all plentiful in Chaulai (Sarker *et al.*, 2020). It also contains lysine, an essential amino acid that is commonly lacking in starch-based diets like cereals and tubers (Maseko *et al.*, 2015).

II.MATERIALS AND METHODS

2.1Collection of plant material and extract preparation

Fresh leaves of Kasuri methi and Chaulai were obtained from local market of Nagour, Rajasthan, and New Delhi, India and identification was verified by Horticulturist at the FICCI Research & Analysis Centre (FRAC) lab in New Delhi. Firstly, the fresh leaves were washed, rinsed, and shade-dried for three days before being pulverised into a coarse powder and stored in airtight plastic container for subsequent study. In a 200ml conical flask, 20g of leaf powder was retained, and 100ml of distilled water was added. For thorough mixing, the tip of the conical flask was covered with aluminium foil and placed in a reciprocating shaker for 25 minutes at 150 rpm. The extracts were filtered with muslin cloth and Whatman filter paper No. 42 (125mm) and kept at 18°C in a screw-capped amber vial (Agarwal *et al.*, 2017).

2.2DETERMINATION OF ANTIOXIDANTS CONTENT

2.2.1Determination of total phenolic content:

Total phenol content was determined using the Folin-Ciocalteu Reagent with gallic acid as a reference phenolic component. Folin-Ciocalteu reagent (5 ml, 1:10 dilution with distilled water) was mixed with a dilute leaf extract (0.5 ml, 1:10 g/ml) or Gallic acid and agitated vigorously. After 3 minutes, 4 ml of aqueous sodium carbonate (1M) was added and the mixture was left to stand for 2 hours with intermittent shaking. Following that, the absorbance was considered in a spectrophotometer at 765 nm against a blank containing all of the reaction values are represented in terms of Gallic acid equivalent (mgGAE/100g dry mass) (Kim *et al.*, 2019).

2.2.2Determination of total flavonoids content

An aluminium chloride colorimetric assay was used to determine the total flavonoids content (TFC). A total of 125 μ l of leaf extract were combined with 75 μ l of a 5% NaNO₂ solution. For 6 minutes, the mixture was left to sit. After that, it was filled with 150 μ l of aluminium trichloride (10%) and incubated for 5 minutes before being filled with 750 μ l of NaOH (1M). Using distilled water, the final volume of the solution was adjusted to 2500 μ l. The liquid coloured pink, after 15 minutes of incubation, and the absorbance was measured with a spectrophotometer at 510nm. The total flavonoids content was calculated in milligrammes of quercetin equivalent (mgQE/100g dry mass) (Gupta and Chaturvedi, 2021).

2.2.3Determination of β carotene

The stock solution of β -carotene was prepared as follows: 0.02 ml linoleic acid and 0.2 ml 100% Tween 20 were added to 0.2 mg β -carotene dissolved in chloroform. A rotary evaporator was used to evaporate the chloroform, and 100 ml of distilled water was added to the residue. The extracts (1 mg/ml) were combined with the emulsion (24 ml) and the first absorbance was measured at 470 nm using a spectrophotometer. After a 2-hour incubation period at 50°C, the absorbance of the reaction mixture was measured once more (Kaska and Mammadov, 2018).

2.24 Determination of Ascorbic acid

To keep the sample's ascorbic acid concentration stable, 10g leaf powder was crushed in 40ml of metaphosphoric acid. Using 6% metaphosphoric acid, the content was prepared up to 100ml. In a conical flask, 20 ml of standard ascorbic acid solution was titrated against 2, 6-dichlorophenol indophenols solution. The completion of the titration was recognized by a faint pink colour resisting for at least 15 seconds (Dinesh *et al.*, 2015).

2.3 DETERMINATION OF ANTIOXIDANT ACTIVITY

2.3.1 Determination of FRAP (Ferric Reducing Antioxidant Power) radical scavenging activity):

In the presence of TPTZ, antioxidants reduce Fe³⁺ to Fe²⁺, resulting in a vivid blue Fe²⁺ -TPTZ complex with a 750nm absorption maximum. pH is a factor in this process (optimum pH 3.6). The reaction mixture is held in a water bath at 50°C for 20 minutes after adding 0.1ml extract to 3.0ml FRAP reagent (10 parts 300mM sodium acetate buffer at pH 3.6, 1 part 10mM TPTZ (2, 4, 6- tripyridyl-s-triazine) in 40mM HCl, and 1 part 20mM FeCl₃). At 595nm, the absorbance was measured. As a positive control, FeSO₄ (100 to 1000 µM ml⁻¹) was used (Jung *et al.*, 2011).

2.3.2 Determination of DPPH radical scavenging activity:

A spectrometric technique was used to analyse the potential of the aqueous extracts to scavenge free radicals against the relatively stable free radical DPPH (2, 2-diphenyl-1-picrylhydrazyl). Aliquots of the sample extract were added to 1 mm aqueous DPPH solutions at varied concentrations ranging from 20 to 200 µg/ml. Each concoction was violently vortexed and left at room temperature in the dark for 30 minutes. The activity was represented as a percentage and the absorbance was calculated at 517 nm. The graph was used to decide the IC₅₀ value (Sandiya and Munniappan, 2015).

2.4 Statistical Analysis:

The results were analyzed using a Mean± SD and Student t-test of three determinations and statistical significance was determined at the p ≤ 0.05 level.

III. RESULTS AND DISCUSSION:

Table 1: Total Phenols and Total Flavonoids content of aqueous of *Kasuri methi* and *Chaulai* leaf powder

Aqueous extract of leaf powder	<i>Kasuri methi</i>	<i>Chaulai</i>	Percentage decrease and increase
Total Phenols (mg GAE/100g)	295.24±0.45	298.61±1.19 ^{NS}	1.14% ↑
Total Flavonoids (mgQE/100g)	250.13±0.85	252.40±0.01 ^{NS}	0.90% ↑

Values are expressed as mean± SD. * denotes significant differences; NS- No Significant difference at p≤0.05 level when compared

Phenolic compounds have been linked to a variety of biological functions and can be found in both edible and non-edible plants. Because of their electron-donating characteristics, phenols can scavenge reactive oxygen species (Rawat and Chaturvedi, 2018). Total phenols content (TPC) (mgGAE/100g) and total flavonoids content (TFC) (mgQE/100g) in aqueous extracts of *Kasuri methi* and *Chaulai* leaf are shown in Table 1. TPC of *Kasuri methi* and *Chaulai* were found to be 295.24±0.45 and 298.61±1.19mgGAE/100g respectively. A similar data was observed by Narzary *et al.*, (2016) who found that the *E. fluctuans* leaves had 269.49 ± 2.96mgGAE/100g and Basumatary and Narzary (2017) also stated that TPC of *M. perpusilla* leaves contained 239.62 ±54mgGAE/100g. The data indicated that TPC of *Chaulai* aqueous extract was insignificantly increased by 1.14% at the p≤0.05 level when compared to *Kasuri methi* aqueous extract.

According to Kumar and Pandey (2013), Flavonoids are the most frequent group of polyphenolic chemicals in the human diet and are ubiquitously present in plants, with a benzo—pyrone structure and are easily eaten by humans and appear to have significant anti-inflammatory, anti-allergic, and anti-cancer characteristics. Total Flavonoids Content (TFC) for *Kasuri methi* and *Chaulai* were 250.13±0.85 and 252.40±0.01mgQE/100g

respectively. Likewise similar data was observed by Shukla and Chaturvedi (2016) that TFC of *Nelumbo nucifera* leaves had 245.22 ± 1.51 . According to Sarker and Oba, (2019) who reported that TFC of *red amaranth* leaves contained 171.26 ± 0.27 and 165.80 ± 0.27 respectively which was comparably lower as contrast to present study. The data indicated that Chaulai was insignificantly increased by 0.90% when compared to Kasuri methi aqueous extract at the $p \leq 0.05$ level.

Table 2. β Carotene and Ascorbic acid of *Kasuri methi* and *Chaulai* Aqueous Extract

Aqueous Extract of leaf Powder	Kasuri methi	Chaulai	Percent decreased and increased
β carotene(mg/100g)	12.10 \pm 0.10	9.08 \pm 0.06*	24.95% ↓
Ascorbic acid (mg/100g)	23.13 \pm 0.07	20.14 \pm 0.18*	12.92% ↓

Values are expressed as Mean \pm SD of triplicate determinations. * Shows significant difference; and ^{NS} shows non-significant difference at ($p < 0.05$) level. Data in parenthesis is % Increased and Decreased

Table 2 shows that β carotene and ascorbic acid contents (mg/100g) of aqueous extracts of Kasuri methi and Chaulai. β -carotene is a key precursor for vitamin A and it also aids in the maintenance of good vision and the treatment of degenerative diseases (Jeyakodi *et al.*, 2018). β carotene of Kasuri methi and Chaulai were 12.10 ± 0.10 and 9.08 ± 0.06 mg/100g and ascorbic acid were 23.13 ± 0.07 and 20.14 ± 0.18 mg/100g respectively. The data showed that Chaulai was significantly reduced by 24.95% and 12.92% at the $p < 0.05$ level when compared to Kasuri methi. The data was comparable with the study reported by Ejoh *et al.*, (2021) who stated that *Solanum americanum* leaves had 10.7mg/100 β Carotene whereas *Amaranthus viridis* leaves had 29.7mg/100g ascorbic acid. Otitoju *et al.*, (2014) also reported that *Psychotria sp.* leaves had (6.64 ± 1.78 mg/100g) β -carotene and (20.00 ± 3.26 mg/100g) ascorbic acid. Vitamin C, commonly known as ascorbic acid, is essential for human development at various phases and, being a powerful reducing agent, it is significant in absorbing and deactivating free radicals, protecting the body from damaging effects (Islary *et al.*, 2016).

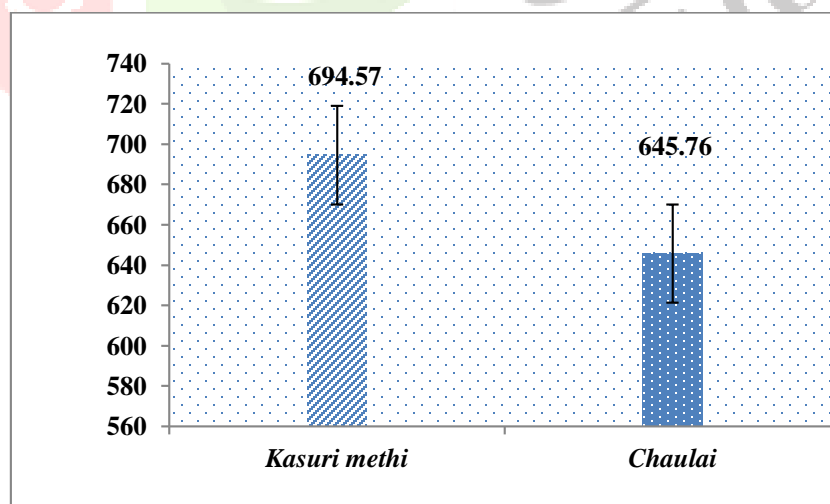


Figure 1.a FRAP (Ferric Reducing Antioxidant Power) radical scavenging activity Analysis in Kasuri methi and Chaulai leaf Extract on Dry Weight Basis

The FRAP assay is used to assess the antioxidant activity, and the reaction is repeatable and directly related to the antioxidant molar concentration (Lewoyehu and Amare, 2019). The Ferric Reducing Antioxidant Power (FRAP) activity (mmolFe²⁺/100g) of aqueous extracts of Kasuri methi and Chaulai leaf were 694.57 ± 1.56

and 645.76 ± 1.86 as depicted in Figure 1.a. At the $p \leq 0.05$ level, the findings showed that Chaulai had significantly decreased by 7.02% when compared to Kasuri methi. A similar study of Rana *et al.*, (2019) who found that FRAP of *Achyranthes aspera* and *Vitex negundo* leaf extract had 505.19 ± 1.56 and 554.41 ± 2.38 FRAP values respectively. The results obtained comparably higher as compared to *S. asper* and *S. oleraceus* leaves as 298.56 ± 32.52 and 201.3 ± 28.72 reported by Jimoh *et al.*, (2011)

Table 3: DPPH Free Radical Scavenging Activity of Kasuri methi and Chaulai leaf powder on Dry Weight Basis

Concentration ($\mu\text{g/ml}$)	Ascorbic Acid	<i>Kasuri methi</i>	<i>Chaulai</i>
20	17	45.1	43.6
40	27	56.8	52.21
60	39.28	65.1	63.1
80	57.47	75.7	71.1
100	74.07	83.5	79.4
200	83.33	92.1	85.7
IC₅₀ Values($\mu\text{g/ml}$)	12.6	15.3	26.04

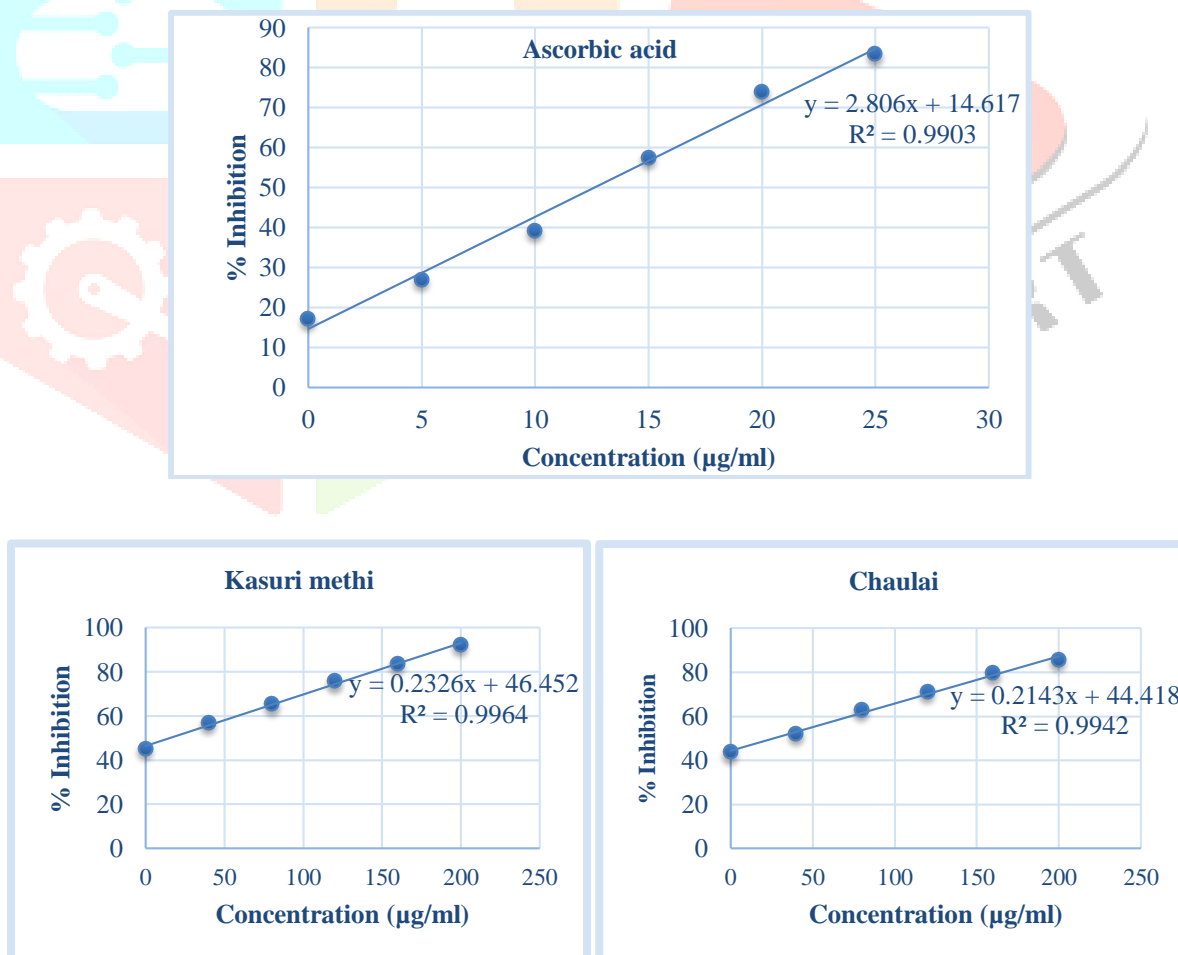


Figure 1.b: The DPPH scavenging activity of *Kasuri methi* and *Chaulai* leaf extract on Different Concentration

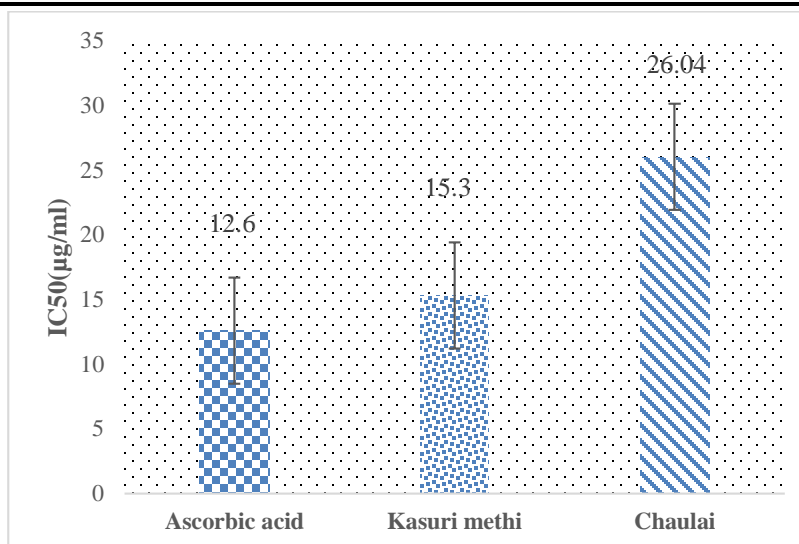


Figure 1.c: IC₅₀ values of DPPH free radical scavenging activity of Ascorbic acid, *Kasuri methi* and *Chaulai* leaf extract

The DPPH radical scavenging activity for ascorbic acid, Kasuri methi and Chaulai methanol extract is shown in Table 3 and Figure 1(b and c). The capacity of biological samples to convert the 1,1-diphenyl-2-picryl hydrazyle radical to 1,1-diphenyl-2-picryl hydrazine is measured in this experiment, hence a decrease in purple colour indicates a decrease in free radicals (Willis *et al.*, 2019). The activity was calculated by comparing the percent inhibition of DPPH radical formation by the extracts to the % suppression of DPPH radical production by ascorbic acid, which served as a positive control. The radical scavenging activity of the control and sample extracts increased when the concentration was increased, and a lower IC₅₀ value implies stronger antioxidant activity. The data pointed out that Kasuri methi aqueous extract exhibits significantly higher antioxidant capacity with IC₅₀ value (15.3µg/ml) whereas Chaulai extract illustrated low scavenging activity 26.04µg/ml in comparison to control (12.6 µg/ml). The results obtained for the present study comparably higher as contrast to *Amaranthus cruentus* leaves as (38.31 µg/ml) reported by Ade-Ademilua and Eyemi, (2013).

IV.CONCLUSION

It can be concluded that both underutilized leafy vegetables (*Kasuri methi* and *Chaulai*) have excellent antioxidant quality. Based on the present data, *Kasuri methi* leaf aqueous extract had significantly higher antioxidant content as well as DPPH scavenging activity when compared to extract of *Chaulai* leaf. Thus, the above findings indicated that *kasuri methi* can be a valuable source of antioxidants in combination of *Chaulai* that can be exploited for the production of nutraceuticals or alternatively, they can be used as a functional food ingredient.

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