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ASSESSMENT OF TOTAL FLAVONOID AND POLYPHENOL CONTENT OF DIFFERENT PARTS OF *CICHORIUM INTYBUS* L.

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ABSTRACT: Common chicory (*Cichorium intybus* L.) plant belonging to sunflower family primarily grown for its root which is used as a coffee substitute but is also widely used in folk medicine to treat various ailments ranging from wounds to diabetes. The plant is a good tonic cooling agent and is useful in headache, throat inflammation, immune stimulation, mutagenic, probiotic, hepatoprotective, antibacterial activity *etc*., and these actions are attributed to a wide range of phytoconstituents present in this plant. In the existing study, the total flavonoid and phenol content of methanolic extract of selected parts (leaves, root, and seeds) of *Cichorium intybus L*.was quantitatively estimated using aluminium chloride colorimetric method (as quercetin equivalent) and Folin-Ciocalteu method (as caffeic acid equivalent) respectively. The total phenolic contents varied from 1.94± 0.895 to 1.66± 0.506 mg/gdw. Total flavonoid contents were between 1.60± 1.69 to 1.37 ± 0.796 mg/gdw.

INTRODUCTION: *Cichorium intybus* L. (Asteraceae) is a bushy perennial herb also known vernacularly as kasani or chicory is grown for its leaves as fodder and root, ground roast powder of which is used as a coffee substitute. Chicory is found to be effective in jaundice, asthma, gout, and rheumatic complaints [1]. Chicory also possesses anti-cancer, antifungal and anti-malarial**,** anti-diabetic, hepatoprotective, and free radical scavenging activity [2-7]. When added to coffee, it neutralizes caffeine and helps in digestion, and also enhances the flavor. Inulin from the root is being used as a substrate of fiber in health and functional foods [8]. Owing to these medicinal attributes, this plant holds a great value for phytochemical analysis.

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Plants and plant-derived products are part of the healthcare system since ancient human civilization [9]. Herbal remedies are currently very popular remedies for diseases used by the majority of the world's population. Recent research on bioactive compounds of medicinal plants like flavonoids, alkaloids, tannin, steroids, glycosides, phenols, carotenoids, benzoic acid, cinnamic acid, folic acid, fixed oils, which are stored in their specific parts of leaves, bark, flowers, seed, fruits, root etc has received much attention [10].

There is an increased evidence for the participation of free radicals in the etiology of various diseases like cancer, cardiovascular diseases, diabetes, autoimmune disorders, neurodegenerative disease, aging *etc*. Any atom or molecule possessing unpaired electrons is defined as a free radical. Antioxidants are agents which scavenge the free radicals and prevent the damage to lipids, carbohydrates, proteins, enzymes, DNA caused by reactive oxygen species (ROS). Antioxidants can greatly reduce the damage due to oxidants by

neutralizing the free radicals or ROS before they can attack the cells. A wide variety of antioxidants from both natural and synthetic origins have been discovered for use in the treatment of various human diseases [11].

Natural antioxidants have become the target of a great number of research studies in finding the sources of potentially safe, effective, and cheap antioxidants. Different studies have indicated the relationship between plant antioxidants and the reduction of chronic diseases. It has been found out that plants having polyphenolic compounds such as flavonoids possess antioxidant activity and have aroused considerable interest recently because of their potential beneficial effects on human health in fighting diseases [12].

Previous research indicates that the biological actions of these compounds are related to their antioxidant activity. The potential of flavonoids to act as antioxidants is credited to their molecular structure. Quercetin, the most abundant dietary flavonol, is a potent antioxidant because it has all the right structural features for free radical scavenging activity [13]. Therefore, the objective of our present study is to determine the polyphenol and total flavonoid content of different plant part extracts of *Cichorium intybus* L. using Aluminium Chloride colorimetric method and Folin-Ciocalteu method. In the study quercetin and caffeic acid are taken as a standard flavonoid and phenol respectively.

MATERIALS AND METHODS:

Plant Material: *Cichorium intybus* L. plant samples were collected from the fields of Nadiad district, Gujrat. 30 kg of plant materials were identified taxonomically by an expert taxonomist at the herbarium, Department of Botany, University of Rajasthan, India. The collected sample specimen no. RUBL211705 has been deposited in the institution herbarium for future reference. Plants were washed with distilled water and then shade dried. Dried plant material were ground into fine powder using a mechanical grinder and kept in airtight container for further analysis.

Estimation of Total Flavonoid Content by Aluminium Chloride Colorimetric Method:

Principle: Formation of acid-stable complexes with the C-4 keto group upon addition of aluminium chloride with the C-3 or C-5 hydroxyl group of flavones and flavonols. Acid labile complexes are also formed by aluminium chloride with the ortho-dihydroxyl groups in flavonoids. For building the calibration curve, quarcetin is used as a standard material. Various concentrations of standard quercetin solution were used to make a standard calibration curve [14].

Procedure: Here, quercetin was used to make the calibration curve. 10 mg of quercetin was dissolved in methanol and then diluted to suitable concentrations. A calibration curve was made by measuring the absorbance of the dilutions at 415 nm (λ_{max} of quercetin) with spectrophotometer. Aluminium chloride, 1%, and potassium acetate, 1M solutions were prepared [15-18].

Preparation of Sample Extracts: Sample extracts of each plant part (root, stem, and seed) were prepared using methanol as extracting solvent. 1g of the dried powdered plant material was extracted using 100ml of methanol by soxhlation for 2 days. Crude methanolic extract was obtained by evaporating the extract to dryness and stored in a refrigerator for further analysis.

Stock Solution of Extracts: 100 mg of each extract was accurately weighed and transferred to 5 ml volumetric flask and made up the volume with methanol.

Preparation of Test Solutions: To each extract solution of 0.50 ml quantity were added 1.5 ml methanol, 0.10 ml aluminium chloride, 0.10 ml potassium acetate solution, and 2.8 ml distilled water. Aluminium chloride with distilled water replacing the plant sample in extract solution was used as blank. Blank and all three sample extracts were prepared, and their absorbance was measured at 415 nm *via* UV-Vis's spectrophotometer. All prepared solutions were filtered using a Whatman filter paper before measuring.

Estimation of Total Phenolic Content by Folin–Ciocalteu Method:

Principle: Folin–Ciocalteu reagent, a mixture of phosphotungstic ($H_3PW_{12}O_{40}$) and phospho- molybdic (H_3PMo_{12}, $H_3PMo_{12}O_{40}$) acids, is reduced to blue oxides of tungsten (W_8O_{23}) and molybdenum (Mo_8O_{23}) during phenol oxidation. This reaction, occurring under alkaline conditions, is carried out in the presence of sodium carbonate. Blue

coloration is monitored at 726 nm and reflects the quantity of phenols, usually expressed as gallic acid or caffeic acid equivalents [19].

Procedure: Estimation of total phenol content in each sample included the preparation of a regression curve of standard phenol (Caffeic acid). A stock solution of caffeic acid was prepared by mixing 40 mg of standard phenol in 1 mL of 80% ethanol. Eight concentrations ranging from 0.1 to 0.8 mL were prepared in the test tube, and volume was raised to 1mL by addition of 80% ethanol. To each test tube, 1mL of Folin-Ciocalteau reagent and 2 mL of 20% sodium carbonate solution were added, and the mixture was shaken thoroughly. The samples were placed in boiling water for 1 min and cooled under running water. These reaction mixtures were diluted to 25 mL by adding distilled water, and optical density was read at 750 nm against 80% ethanol as blank. The optical density of each sample was plotted against the respective concentration of total phenols to compute the regression curve. The concentrations in the test samples were calculated by referring to the respective optical density of the test sample against the standard curve of caffeic acid.

Preparation of Test Solutions: 0.2 gm plant sample was crushed in 3 ml of 80% ethanol. The mixture was centrifuged at 1500 rpm for 20 mint at RT. Then 1 ml of supernatant was taken in a test tube, and I ml of Folin–Ciocalteu reagent and 2 ml of Sodium Carbonate were mixed. OD was measured by a spectrophotometer at 750 nm. 80% methanol was set as blank.

RESULTS AND DISCUSSION:

Determination of Total Flavonoid Content: Flavonoids are plant secondary metabolites widely distributed in the plant kingdom. **Fig. 1** shows the standard calibration curve of quercetin (0.1mg/ml stock solution) for the determination of total flavonoid content in the methanolic extracts of different plant parts. Quercetin standard curve was used for computing the concentration of quercetin equivalent present in the extracts, by interpolating to the X-axis. TFC was calculated by using the following formula [20].

Total Flavonoid Content = R ×D.F×V×100 / W

Where, R- Concentration computed through standard curve of quercetin. V - Volume of stock Solution. D.F. - Dilution factor. 100- for 100 gm dried Plant. W - Weight of the plant used in the experiment (in gm).

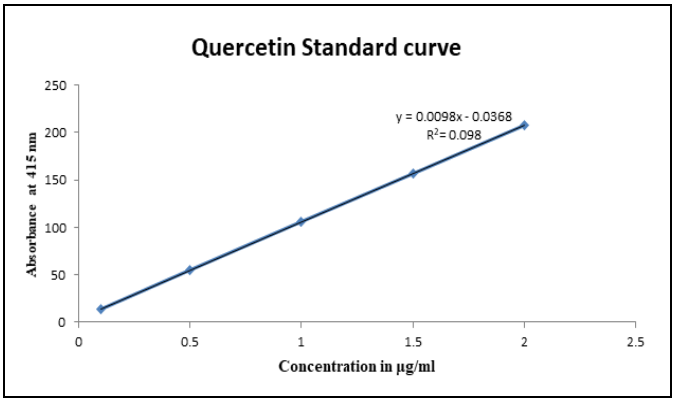

FIG. 1: STANDARD CURVE OF QUERCETIN (0.2 mg/mL STOCK SOLUTION

Determination of Total Phenolic Content: Total phenolic content was determined using the colorimetric method. The concentrations of phenols were expressed in mg/ml unit. Quantification was done on the basis of a standard curve of caffeic acid **Fig. 2.** Results expressed as percentage w/w and calculated using given formula [21-23].

Total Phenolic Content (%w/w) = CAE×V×D×10^{-6}×100\W

Where CAE-Caffeic acid equivalent (µg/ml). V - Total volume of sample (ml). D - Dilution factor W - Sample weight. Assays were performed in triplicates. Values are expressed as means ± SD.

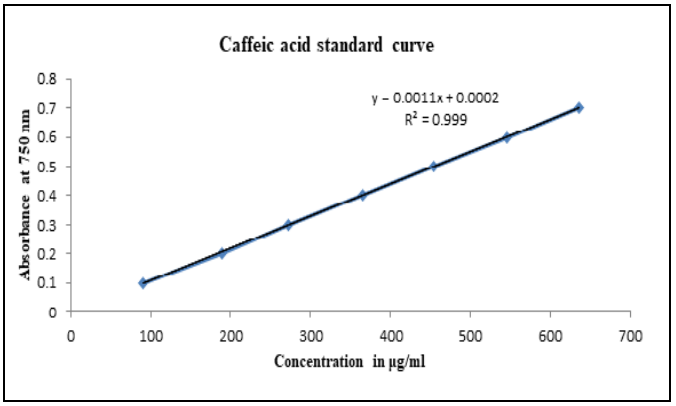

FIG. 2: STANDARD CURVE OF CAFFEIC ACID

Total flavonoid and phenolic content of different plant part extracts is shown in **Table 1**. Total flavonoid content is expressed as quercetin equivalent (QE; µg quercetin/100g) and total phenolic content is expressed as caffeic acid equivalent (CAE; caffeic acid µg/100g).

TABLE 1: RESULTS OF CALIBRATION CURVE

Sample of *Cichorium intybus L.*	Total flavonoid content in mg/100g of dried material (in QE)	Total phenolic content in mg/100g of dried material (in CAE)
Root	140.95±0.959	166.33± 0.506
Leaf	160.742±1.69	194.867±0.895
Seed	137.73±0.796	175.518±0.608

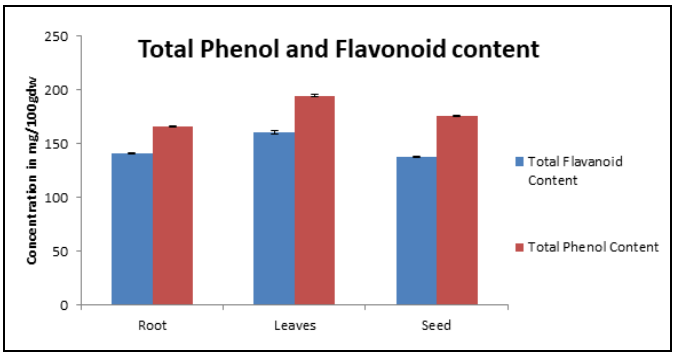

FIG. 3: COMPARATIVE DIFFERENCE FOUND AMONG THE TOTAL PHENOL AND FLAVONOID CONTENT

CONCLUSION: From the results it was observed that *Cichorium intybus* L. leaves contained the highest amount of phenolics and flavonoids while the least amount of phenolics and flavonoids was found in roots and seeds respectively. These data emphasize the pharmacological significance of the plant as the total amount of analysed phytochemicals is considerably high. Further investigations need to be carried out for the isolation and characterization of bioactive compounds and thorough screening of antioxidant activity needs to be performed.

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CONFLICTS OF INTEREST: Nil

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