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Phytochemical parameters of *Cichorium intybus* in methanolic and aqueous leaf and root extract

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Abstract
The phytochemical activity of *Cichorium intybus* (chicory) was investigated through the analysis of methanolic and aqueous leaf and root extracts. Fresh chicory was collected, and leaves and roots were processed for extraction using methanol and water. The extracts were analyzed for Total Phenolic Content, Total Flavonoid Content, and Total Flavanol Content. Methanol extracts showed higher total phenolic content, total flavonoid content, and total flavanol content compared to aqueous extracts. Methanol leaf extract exhibited the highest total phenolic content (116.62±3.53 mg GAE/g extract) and total flavanol content (75.44±0.33 mg QE/g extract). This research highlights the complex and promising phytochemical profile of chicory, paving the way for further exploration and applications in pharmaceuticals and nutrition.

Keywords: Phenol, flavonoid & flavanol

Introduction
The phytochemical analysis of *Cichorium intybus*, family- Asteraceae reveals a diverse array of chemical constituents distributed throughout its various parts [2, 3, 5]. Chicoric acid emerges as the primary compound identified in methanolic extracts, underscoring its significance within this herb [5, 6, 10]. The roots exhibit a rich composition, housing sesquiterpene lactones (lactucin, lactucopicrin, 8-deoxy lactucin, guaiacol glycosides), caffeic acid derivatives (chicoric acid, chlorogenic acid, chlorogenic acid, caffeoyl tartaric acid), inulin, sugars, proteins, hydroxyl coumarins, flavonoids, alkaloids, steroids, terpenoids, oils, volatile compounds, and vitamins [11, 14, 20]. Notably, leaves, flowers, and seeds also contain a diverse spectrum of phytochemicals, including reducing sugars and various compounds akin to those found in the roots [2, 14, 15]. The composition showcases high alkaloid content, dominant aliphatic compounds, minor terpenoids like coumarin cichorine and flavonoids, alongside anthocyanins contributing to the distinctive blue hue of the perianth [3, 41, 4] Furthermore, the roots and leaves of chicory boast a notable abundance of antioxidants and biochemicals, positioning them as potential candidates for pharmaceutical formulations and advocating for their role in fortifying the body's defence against endogenous free radicals [6, 7, 9].

Materials and Methods
Plant Material Collection and Preparation
- Fresh *Cichorium intybus* was collected from the Chakrata region, Dehradun, in March 2017.
- Leaves and roots were meticulously separated, and washed to eliminate foreign particles, and damaged portions were removed.
- The plant parts were dried in shade for around 8 to 12 days, followed by further drying in a hot air oven at 40°C for 14 hours until they were easily crushable into powder.

Extraction Process
- Methanol was used as a solvent for the extraction process. Plant powder (2 grams) was mixed with 30 ml of methanol and shaken overnight at room temperature [9, 10, 18].

- The solution was filtered, and the supernatant was collected. The residue was re-dissolved using ethanol until the green colour disappeared [19].
- The supernatant was evaporated using a rotary evaporator, yielding a stock solution of 10 mg/ml extract in methanol [14].

Percentage Yield of Extracts Calculation

The percentage yield of extracts was determined based on the dry weight sample using the formula:

(Weight of extracts (gm) / Weight of dry plant powder (gm)) x 100.

Phytochemical Analysis

- **Total Phenolic Content:** Estimated using the Folin-Ciocalteu method, involving the reaction of phenolic compounds with Folin–Ciocalteu reagent under basic conditions, measured by absorbance at 725 nm.
- The total Phenolics content was calculated and determined according to the Folin-Ciocalteau method using a calibration curve, and the results were expressed as mg of Gallic acid equivalent (GAE) per gram dry weight of the sample [20].
- **Total Flavonoid and flavanol Content:** Determined using the aluminium chloride colourimetric assay, measured at 510 nm for the flavonoid and 440nm for the flavanol against a standard curve of Quercetin equivalents

Results

Phytochemical content estimation

Cichorium intybus L. is reported to be of high medicinal importance due to phytochemical content. The analysis of aqueous and methanolic extracts of *Cichorium intybus* L. for its phytoconstituents showed that dry leaves of *Cichorium intybus* L are rich in total phenols, total flavonoids and flavanols content. All the studied parts of *C. intybus* are good source of phenolic compounds. The root has been found to show comparatively low phytochemical activities due to the low content of total flavanol content and total phenolic content. However, methanolic extracts of *C. intybus* leaves possess comparatively higher amounts of total flavanol content, and total phemolic content.

Total Phenolics Content: *C. intybus* has been found to have great medicinal importance due to the presence of phenolic compounds. Considering [7, 10, 23] methanol is most efficient and widely used to extract anti-oxidative components including phenolic acids and other phenolic components among other different fractions of solvents. Although water also extracts reasonable amounts of total phenolic content, however, due to comparatively lower polarity, is less effective.

Methanol extract of the *C. intybus* leaves and roots showed the highest total phenolic content respectively among those aqueous leaf and root extracts. The amounts of total phenolic content from *C. intybus* leaves and roots in different solvent systems were in the ranges of 116.62±3.53 and 94.5±0.72 mgGAE/ g extract in methanolic leaf and root respectively. Total phenolic content was calculated as mg Gallic acid equivalent/ g extract by applying the linear regression equation obtained from a calibration curve (R2 = 0.996).

Table 1: Total phenol content, total flavonoid content and Total Flavanol content in Methanol and Aqueous extracts of leaves and root parts of *C. intybus*.

Phytochemical	Methanol leaf extract	Methanol root extract	Aqueous leaf extract	Aqueous root extract
Total Phenol Content (mg GAE/ g extract)	116.62±3.53	94.5±0.72	70.45±0.60	48.3±0.37
Total Flavonoid Content (mg Quercetin equiv./g extract)	20.55±0.32	1.11±0.00	7.22±0.32	2.22±0.64
Total Flavanols Content (mg Quercetin equiv./g extract)	75.44±0.33	50.14±0.33	63.97±0.59	45.73±0.33

Relating to methanol extract of leaves contains the highest total phenol content (62.5mg GAE / g extract) and the aqueous extract of roots contains the lowest total phenolic content (23.4 mg GAE/g extract) giving a significant data highlighting that methanol extract of the *C. intybus* leaves and roots showed the highest total phenolic content respectively [12]. These differences for total phenolic content may be due to varied efficiency of the extracting solvents to dissolve endogenous compounds. The ability of different solvents to extracts total phenolic content was of the order: Methanol > Chloroform > Aqueous. Starting with the better extract, the amount of total phenolic content in *C. intybus* follows the order Methanolic leaf > Methanolic root > Aqueous leaf > Aqueous root. And hence so far Polyphenolic compounds have attained great attraction from investigators because of their antiviral, antifungal, antibiotic, antitumor, anti-inflammatory, antimutagenic and antioxidant activities [14, 20].

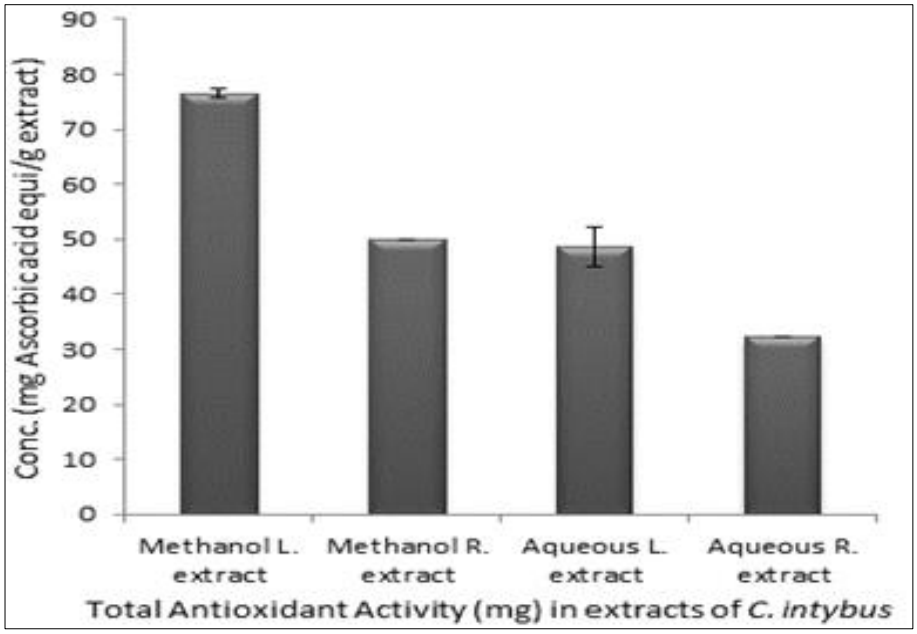

Fig 1: Total Phenolic Content in leaf and root extracts of *C. intybus.*

Total Flavonoid Content

The flavonoids and flavanols are known to possess antioxidant activities due to the presence of hydroxyl groups in their structures and their contribution to the defense system against oxidative damage due to endogenous free radicals is extremely important [131-16].

The extract was found to be rich in high flavonoid content, which may be responsible for its observed antioxidant activity. The methanolic extracts of leaf and root have been found to possess more Flavonoid as well as Flavanol content as compared to the aqueous extracts of *C. intybus.* The maximum total phenolic content in the methanolic extracts of leaf and root are 20.55±0.32 and 1.11±0.00 respectively. In contrast, aqueous solvent extracts show slightly less total flavonoid content on comparison i.e., 7.22±0.32 and 2.22±0.64 mg of Quercetin equivalent/g extract in leaf and root part respectively as shown in table number 1.

These observed activities can be explained by the properties of the isolated compounds and phytoconstituents present in the extract, such as flavanols and flavonoids. Plants contain a mixture of many kinds of secondary metabolism products, including phenols, which vary greatly in their antioxidant capacity. Flavonoids have anti-inflammatory, anti-allergic, anticarcinogenic, antioxidant, and antiviral properties [5].

Thus starting with the best total flavonoid content extraction follows the order: Methanolic leaf > Aqueous leaf> Methanolic root > Aqueous root.

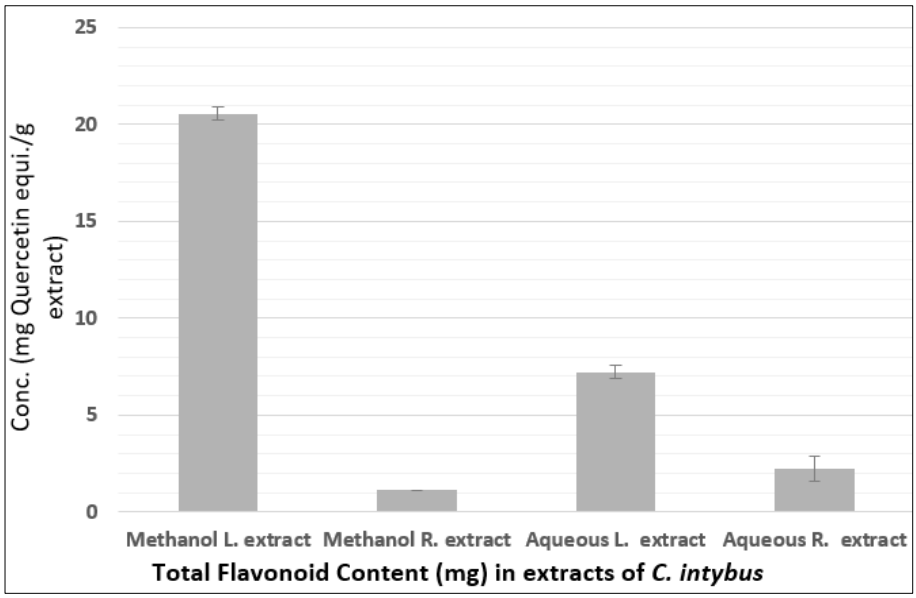

Fig 2: Total Flavonoid Content in leaf and root extracts of *C. intybus.*

Total Flavanol

Like Total Flavonoid Content, the Total Flavanol content estimation too takes mg Quercetin equivalent/ g extract (QE) as standard. In fig 4.1.3, the flavanols estimation of methanolic and aqueous extracts in leaf and root parts of *C. intybus* is been compared. Here, methanol leaf possesses maximum flavanol content (75.44±0.33 mg Quercetin equivalent/g extract) as compared to aqueous extracts (fig.4.1.3). The significant order follows as methanol leaf> aq

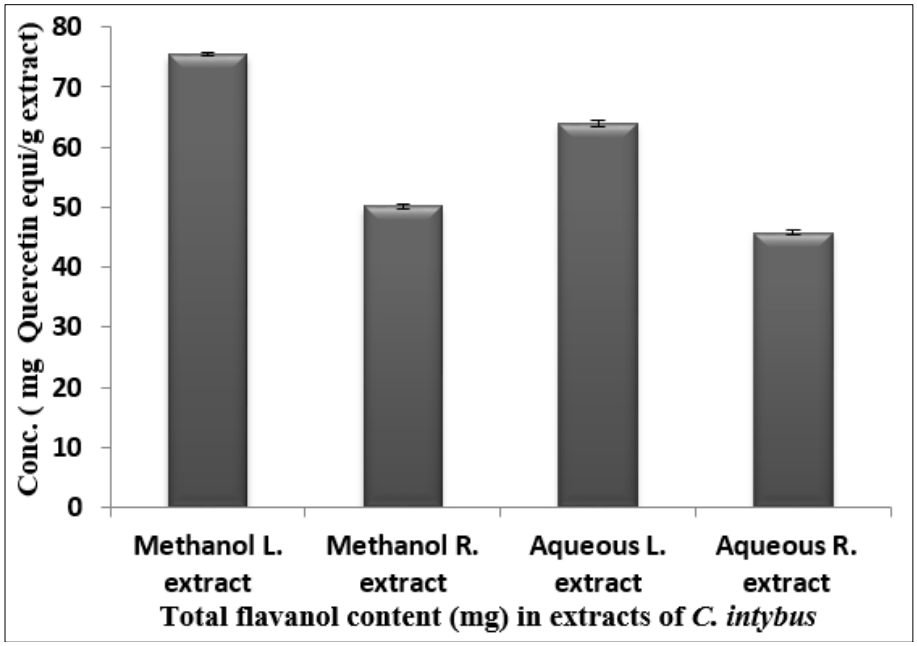

Fig 3: Total Flavanol content in leaf and root extracts of *C. intybus*.

ueous leaf> methanol root> aqueous root. Root part of both solvents tends to show lower flavanol content i.e., 50.14±0.33 and 45.73±0.33 in methanol and aqueous root part respectively. Hence leaves show a better perspective towards total flavanol and so have such multiple biological effects as anti-atherogenic, antioxidant, anti-inflammatory, cardioprotective, antimicrobial, anticarcinogeni and neuroprotective. (Refer table number 1)

The chicory leaf extract used in this study was partially described with reference to total phenolic and total flavonoids compounds and its maximum extraction in methanol as a solvent. Therefore, the complex of phytochemical active substance in dry leaves of *Cichorium intybus* L. offers many opportunities for future application in herbal medicine and nutrition industry to produce healthy food.

Conclusion

The study on *Cichorium intybus* revealed significant levels of phytochemicals in all examined parts, signifying its role as a robust source of natural compounds. Notably, methanol extracts, particularly from the leaves, exhibited heightened concentrations of these compounds. The choice of solvent significantly impacted the extraction efficiency, with methanol demonstrating superior extraction of phenolic compounds compared to aqueous extracts from both leaves and roots. Specifically, the methanol leaf extract showcased the highest phenolic content, while the aqueous root extract displayed the lowest. Moreover, methanol leaf extracts showed elevated levels of Total Flavonoid Content, Total Flavanol Content & total phenolic content.

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