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Phytochemical screening and quantitative analysis of *Cichorium intybus* L. (Chicory) plants from region of Uttarakhand

Mamta Arya, Brij Raj Singh and Gohar Taj

Abstract
Cichorium intybus (chicory) plant contains various secondary metabolites like other plants with proficient potentials. The purpose of this research is to find out an appropriate extraction solvent system of methanol, chloroform, ethanol, hydro-ethanol, and acetone extracts for analysis of various phytochemicals with the aid of standard techniques. The findings from phytochemical screening and quantification depicted the presence of phenol, flavonoids, saponin, tannin, alkaloids, protein, glycosides, and carbohydrates. Furthermore, the study findings showed that methanol extract of chicory leaf extract was found to have more bioactive components when compared with the other extracts by quantitative technique. Among the five different extracts of leaf chicory saponin and phenol were maximum in methanol extract. Whereas, flavonoids were found maximum in ethanol extract as compared to other constituents. Alkaloids and tannin were found maximum in ethanol and hydro-ethanol extract respectively. A good phytochemical composition of *Cichorium intybus* L. leaves would be an essential contender in pharmaceutical articulations and play a significant role in ameliorating human health by participating in the antioxidant defense system.

Keywords: *Cichorium intybus* L., therapeutic drugs, phytochemical constituents, qualitative screening, quantitative estimation

1. Introduction
Biologically active compounds from herbal sources have been of great interest for researchers employed on infectious diseases (Perumal *et al*., 2000) [22]. *Cichorium intybus* L. is a therapeutically foremost plant that refers to the family Asteraceae. The leaves of the plant have many traditional uses with a lot of medicinal benefits. Chicory leaf has distinctive dietary fiber sources that have favorable properties as fiber ingredients for poultry nutrition (Liu *et al*., 2013) [16]. Its use is also in wound healing (Sezik *et al*., 2001) [21]. *Cichorium intybus* leaf extract is used as a safe growth promoter, immune stimulator, and hepato-protective in broiler production (Saeed *et al*., 2015) [27].
The leaf extract of *C. intybus* showed moderate activity against multidrug resistant *Salmonella. Typi* (Rani *et al*., 2004). Its leaves contain phosphates and sulfates salts of sodium, potassium, and magnesium as well as potassium nitrate. Adequate amount of cichorine, a bitter glycoside also present in leaves of chicory. Chicory leaves are used as laxative, diuretic, antipyretic, anti-bilious, and help in blood purification and nourishment of the stomach. Other uses of chicory leaves are as an appetizer, treatment of hepatic failure, jaundice, spasmodic fever, and mild states of chronic skin diseases (Ghaderi *et al*., 2012) [8]. Leaves of chicory are also useful in the treatment of diabetes. Ethanolic extract prepared from leaves of chicory was estimated for preventive actions on free radical arbitrate damage to the deoxyribose sugar of the DNA.
Chicory leaves are used as an ointment for wound healing especially in Turkey (Sezik *et al*., 2001) [29]. Chicory leaves aqueous-alcoholic macerate shows an anti-proliferative effect on cell lines of amelonotic melanoma C32 (Conforti *et al*., 2008.). In post-harvest period of chicory crop, the essential ployamines are found as putrescine, mainly in the matured leaves, while spermidine is present in substantial amounts, indicating a tendency to reduce with the rising physiological age of the leaves. Also with the physiological age of the leaves and its postharvest period, there is an increase in the free sterol content. The major free sterol present is sitosterol, followed by campesterol and stigmasterol (Krebsky *et al*., 1999) [12]. A variety of chemicals are present in chicory leaves which are useful for human health. The main aim of the present investigation is to extract the different phytochemicals of maximum concentration. For that different kinds of extraction solvents were tested.

2. Material and Methods

2.1 Plant Material Collection and Identification

The chicory plant was collected from the Pithoragarh district of Uttarakhand, India. The authenticity of the plant was confirmed by the Department of Botany, CBSH, GBPUAT, Pantnagar. The leaves of chicory were washed with distilled water and wiped with 70% ethanol. The washed leaves were dried at room temperature and which was used for the preparation of the plant extract. The plant extracts were used for performing the qualitative and quantitative analysis.

2.2 Preparation of Extract

The *Cichorium intybus* plants powder was extracted with various solvents. 5 gm of dried powder of chicory was suspended in 100ml of each acetone, methanol, ethanol, chloroform, hydro-ethanol (50:50) solvents. Extraction was done using the maceration method and kept for 2 days and filtered the extract using muslin cloths (Roghini *et al*., 2018) [26]. Then, the filtered extract was dried, and after dried extracts were collected and weighed for quantification of yield and extraction yield percentage. Moreover, the extract has persevered in a dried plastic container for further analysis (qualitative and qualitative analysis) (Roghini *et al*., 2018) [26].

2.3 Qualitative Phytochemical Screening

The phytochemical screening process was carried out to estimate the secondary metabolites which are found in the chicory plant. Therefore, these secondary metabolites present in the different extract of leaves of *Cichorium intybus* plant was analyzed through qualitative analysis (Roghini *et al*., 2018) [26].

2.4 Quantitative Estimation of Secondary Metabolites:

2.4.1 Estimation of saponin

To determine saponin content of *Cichorium intybus* extract Vanillin-Sulfuric acid assay of Hiai 1976 was adopted. 0.5 ml of aqueous sample solution, 0.5 ml 8% (w/v) vanillin solution, and 72% (w/v) 5.0 ml of sulfuric acid were added and mixed in an ice water bath. Following this, the mixture was then warmed in a water bath at 60 ˚C for 10 min after that it was cooled in ice-cold water. For estimation of saponin, the standard curve of oleanolic acid equivalent (OAE/g) was used. Absorbance values were measured at 527 nm (Kose *et al*., 2016).

2.5.2 Estimation of alkaloids

Alkaloid was estimated by taking a part residue (1ml of prepared extract) dissolved in 5ml 2N HCL and then filtered. 1ml of this solution was transferred to a separatory funnel and washed three times with 10 ml Chloroform (3 times). The pH of this solution was adjusted to neutral with 0.1N NaOH. 5ml of BCG solution and 5ml of phosphate buffer was added to this solution. The mixture was shaken and complex extracted with 1, 2, 3, and 4 ml chloroform by vigorous shaking, the extract was then collected in a 10 ml volumetric flask and diluted with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80, and 100 µg/ ml) were prepared in the same manner as described already. The absorbance for standard solutions and test solutions were determined on the reagent blank at 470 nm with a UV/Visible spectrophotometer. The content of alkaloids was expressed as mg of AE/g of plant extract (Selvakumar *et al.*, 2019) [28].

2.5.3 Estimation of phenols content

To determine phenolic content 1ml of sample extract was added with 1ml of 95% (V/V) of ethanol followed by the addition of 5 ml of distilled water. To each sample extract, 0.5 ml of 50% (V/V) folin co-chalateau reagent was added and the mixture was left for 3 minutes. Add 2ml of 20% (V/V) Na2CO3 and incubate for 60 min. The absorbance was measured at 650 nm and a standard curve of Gallic acid was used for the quantification of total phenols. The total phenol was expressed as Gallic acid equivalent (mg GAE/g extract) (Chaubey *et al.*, 2017) [2].

2.5.4 Estimation of Flavonoids

For estimation of flavonoids add 1 mg of extract into 1 ml of methanol solvent. 1 ml of extract of different polarity from stock solution was added with 3 ml of ethanol. The volume of 0.2 ml of AlCl2 (10% V/V) was added followed by 0.2 ml of 1M solution of potassium acetate. After adding 2.8 ml of distilled water in each, the mixture was incubated at room temperature for 6 minutes absorbance was taken at 415 nm. Methanol was taken as a blank and a standard curve of catechol equivalent (mg CE/g extract) was used for quantification of flavonoids contents (Hossain and Shah 2015) [10].

2.5.5 Estimation of Tannin

Tannin estimation was performed by taking 0.5 ml of sample extract of concentration of 1mg/ml was taken in a test tube. The volume was made up to 1 ml with the distilled water and 1ml of water serves as the blank. To this 0.5 ml of Folin's phenol reagents (1:2) followed by 5ml of 35% sodium carbonate was added and kept at room temperature for 5min which lead to the blue color formation and the color intensity was read at 640 nm. A standard curve of gallic acid equivalent was plotted, from which the tannin content of the extract was determined. The total tannin content was expressed in mg GAE/g of extract (Sreedevi *et al*., 2017) [30].

2.6 Statistical analysis

The phytochemical components (Total flavonoids, Total phenolic, Tannins, Total alkaloids, saponins) were articulated as the Mean± SD of triplicate reading, and comparisons were implemented by a one-way ANOVA. A value of $p \leq 0.05$ was contemplated statistically significant.

3. Results

3.1 Qualitative Screening

The phytochemical qualitative analysis of various extracts is depicted in Table 1. Initiating with the qualitative screening, it is shown that the *Cichorium intybus* of various extracts confirmed the presence of phenols, alkaloids, flavonoids, saponin, tannin, carbohydrates, glycosides, and proteins. Furthermore, saponin, tannin, and proteins were present in all leaves extract (solvents). Carbohydrates and glycosides were present in all extracts except acetone and chloroform. Phenols and alkaloids were present in all the extracts except in ethanol and acetone respectively. Also, flavonoid content was present only in hydro-ethanol and methanolic extracts. Though, along with these compounds phenolic, flavonoids, tannin, saponin, and alkaloids are essential secondary metabolites and are liable for medicinal values for the particular plant. Moreover, the extract was vanquished to further analytical tests for the quantification of phytochemicals compounds

Table 1: Phytochemical screening of leaf extract: the precursory phytochemical screening of the leaf extracts employing different solvents was recited shown in table 2.

Phytochemicals	Acetone	Chloroform	Ethanol	Hydro-ethanol	Methanol
Alkaloids	-	+	+	+	+
Flavonoids	-	-	-	+	+
Saponin	+	+	+	+	+
Tannins	+	+	+	+	+
Phenols	+	+	-	+	+
Carbohydrates	-	-	+	+	+
Glycosides	-	-	+	+	+
Protein	+	+	+	+	+

3.2 Quantitative analysis

3.2.1 Extraction yield

For each solvent extraction yield of *Cichorium intybus* was measured using the dried extract of chicory with different solvents. These prepared extracts were measured as extraction yield in percentages for individual solvents. Each solvents extraction yield was between 3.5-39.72%. In table 2 it is shown. The highest yield (39.72%) was obtained in Hydro-ethanol solvent and its dilution ratio was 50:50. The yield was lowest (3.5%) in the case of chloroform solvent. The extraction yield in methanol, acetone, and ethanol solvents was 7.22%, 5.34%, and 11.32% respectively depicting that the extraction efficiency suits the highly polar solvents

Table 2: Extraction yield and yield percentage of *Cichorium intybus* in different extraction solvent system

Solvents	Yield (mg)	Extraction yield (Percentage)
Chloroform	175	3.5%
Ethanol	566	11.32%
Acetone	267	5.34%
Hydro-ethanol	1986	39.72%
Methanol	361	7.22%

The quantity of phytochemicals that were detected in the samples of ethanol, hydro-ethanol, and methanol were more abundant whereas in the case of acetone and chloroform were less abundant. These phytochemicals were quantitatively determined by the standard protocols. Among the five secondary metabolites which were quantified, the highest content was found of saponin (257.33±0.170 mg OAE/g) and phenol (302±0.251 mg GAE/g) in methanol extract (Table 3).

3.2.2 Determination of Alkaloids

The total alkaloid contents in the leaf part of *Cichorium intybus* showed that higher alkaloids contents were present in the methanol leaf powder (59.51 mg AE/g) than other solvents as shown in Table 3. Whereas the minimum alkaloids content of 19.78±0.101 mg AE/g was observed in chloroform solvent.

3.2.3 Determination of Flavonoids

The total flavonoids content was high in the case of ethanol extract (226 mg CE/g) as compared to the other extracts (Table 3). However, the minimum flavonoids content of 35.25±0.362 mg CE/g was observed in acetone solvents.

3.2.3 Determination of Saponin

The total saponins content of the leaf part of *Cichorium intybus* was ranging between 40.66 mg OAE/g and 257 mg OAE/g extract through the different solvents extracts studied (Table 3). The methanolic extract of the leaf part of *Cichorium intybus* described high content of saponins 257 mg OAE/g. Whereas minimum saponin content 40.66±0.253 mg OAE/g was observed in hydro-ethanol solvent.

3.2.4 Determination of Tannin

The tannin content of the leaf part of *Cichorium intybus* was detected to be present in all five solvent hydro-ethanol extracts (Table 3). Amongst the solvents used, hydro-ethanol has found a high amount of 297 mg GAE/g of tannin content. On the contrary, acetone leaf extract consists of a lower amount of 62 mg GAE/g of tannin content.

3.2.5 Determination of Phenols

Total phenolic content of different extracts of leaf of *Cichorium intybus* was observed widely ranging from 77 mg GAE/g to 302 mg GAE/g (Table 3). Methanolic extract of the leaf has a signifying higher total phenolic content (302 mg GAE/g) than that of the other solvent extracts. Though, the minimum phenol content was 77±0.033 mg GAE/g in acetone solvent.

Table 3: Total phenol, total Flavonoids, total alkaloids, saponin and tannin content of various extracts of leaf of *Cichorium intybus*

Phytochemical	Acetone	Chloroform	Ethanol	Hydro-ethanol	Methanol
Total alkaloids [1]	43.56±0.051	19.78±0.101	21.40±0.090	38.70±0.090	59.51±0.078
Total Flavonoids [2]	35.25±0.362	85.25±0.055	226±0.063	202.75±0.061	172.75±0.065
Saponin [3]	160±0.112	197.31±0.180	64±0.259	40.66±0.253	257.33±0.170
Tannins [4]	62±0.057	87±0.149	277±0.251	297±0.206	202±0.164
Total phenols [4]	77±0.033	102±0.239	167±0.199	232±0.219	302±0.251

Values were achieved in triplicate and represented as Mean±SD: mg AE/g extract, 1: mg CE/g extract, 2: mg OE/g extract, 3: mg GAE/g extract, 4. Mean values followed by different superscript column are significant with $p \leq 0.05$ value.

4. Discussion

To obtain bioactive compounds from the plant, there are various steps including milling, grinding homogenization, and extraction (Do *et al.*, 2014). Amongst these steps, extraction is the imperative step to retrieve and segregate bioactive compounds from the sources. Efficacy of the extraction is strenuously affected by the extraction process, temperature, composition of phytochemicals, the extraction time, and the solvent used (Turkmen *et al.*, 2006; McDonald *et al.*, 2001, Ngo *et al.*, 2017) [32].

The current study utilized distilled water and organic solvents (methanol, ethanol, hydro-ethanol, chloroform, and acetone) to extract bioactive compounds from *C. intybus* leaves. Results indicate that distinct solvents resulted in various extraction yields. This is due to the difference in the polarity of the extraction solvents could invoke an ample variation in

the level of bioactive compounds in the extract. A higher extraction yield was detected in hydro-ethanol extract, ethanolic extract, methanol extracts in comparison to acetone and chloroform extracts, showing that the extraction efficacy relates to the highly polar solvents. This result is congruent with the extraction yield of *Limophila aromatic* (Do *et al.*, 2014) and a few other medicinal plants (Kuppusamy *et al.*, 2015) [14]. This could be due to the plant material consisting of high levels of polar compounds that are soluble in solvents using high polarities such as water, methanol, and ethanol.In conformity with the extraction yields, the content of bioactive compounds (alkaloids, flavonoids, phenolics, and saponin and tannin) differed amongst the extracts. The utmost levels of alkaloids, saponin, and phenolics were determined in methanolic extracts, thus indicating the highest extraction yield of methanolic extract. This can be imputable to the higher solubility of these compounds in methanol than the other solvents tested (Do *et al.*, 2014).

The plant generates numerous chemical compounds like secondary metabolites through metabolic pathways resulting from primary metabolic pathways. Phytochemical screening of the leaves extract of *Cichorium intybus* reveals the existence of saponin which are steroid or triterpenoid glycosides represented by their bitter or astringent taste, foaming properties, and their hemolytic effect on red blood cells (Buren *et al*., 1969; Prohp *et al*., 2012) [33, 23]. Saponins retinues both valuable (cholesterol-lowering) and harmful (cytotoxic permeabilization of the intestine) properties and also reveal structure-dependent biological activities (Buren *et al*., 1969) [33]. Saponin invokes reduction of blood cholesterol by obviating its reabsorption which creates it useful in cardiovascular diseases. Moreover, it has been authenticated that saponin has antitumor and anti-mutagenic actions and can lower the risk of human cancers, by forestalling cancer cells from growing (Osagie *et al*., 1998) [19].

In the present investigation, the saponin content was maximally present in methanol (257.33 mg OAE/g) and followed by chloroform (197.31 mg OAE/g), acetone (160 mg OAE/g), ethanol (64±0.259 mg OAE/g), hydro-ethanol (40.66±0.253 mg OAE/g), and methanol (40.66 mg OAE/g) extracts. This is in contrast to that of Edewor *et al*. who showed that the leaves of *Cassytha filiformis (linn.)* contain the highest saponin in methanolic extract compared to other phytochemical constituents (Edewor *et al*., 2016) [5]. Saponins can react with cholesterol-fortified membranes of cancer cells, thus preventing their growth and feasibility (Rao *et al*., 1995) [25]. Plants that secrete saponins are capable to fight infections by parasites and in humans, it works as an immune system booster and also defends against bacteria and viruses. The saponins have antioxidant activity and its non-sugar part is directly responsible for the action which may consequence in reduced risk of cancer and heart diseases (Prohp *et al*., 2012) [23]. Flavonoids content estimation was highest in ethanol (35.25 mg CE/g) and trailed by hydro-ethanol (202.75 mg CE/g), methanol (172.75 mg CE/g), chloroform (85.25 mg CE/g), and acetone (35.25 mg CE/g) extract. Similar results were observed in the leaves of *Citrus paradisi* consisting of a maximum quantity of flavonoids in the ethanolic extract in comparison to the other types of phytochemicals (Roghini, R. and Vijayalakshmi, K., 2018) [26]. Flavonoids are commonly distributed secondary metabolites with diverse metabolic functions in plants. These are water soluble phenolic molecules and hence belong to the polyphenol family. These are also responsible for the coloring of vegetables, fruits, and herbs. Flavonoids have better anti-oxidative properties compared to health promoting effects such as vasoprotective, anti-allergic, tumor inhibitory, anti-inflammatory, anti-cancer, anti-oxidant, anti-viral, anti-thrombotic effects. These effects have consorted with the impact of flavonoids on arachidonic acid metabolism. Certain flavonoids containing plants are antispasmodic, diuretics and others have antimicrobial properties (Evans, 2002) [7].

Furthermore, epidemiological research has revealed that heart diseases are contrarily associated with flavonoid intake and that flavonoids avert the oxidation of LDL as a result mitigating the risk for the development of atherosclerosis. The utility of flavonoids as anti-diabetic and hypoglycaemic has been reliable (Tanko *et al*., 2007) [31]. Besides, the effects of flavonoids, ferulic acid, and quercetin on pancreatic-β-cells preeminent to their proliferation and secretion of more insulin have been propounded by as the mechanism of their hypoglycemic activity in streptozotocin-induced diabetic rats (Mahesh *et al*., 2004) [17]. These may make the leaves of *Cichorium intybus* aqueous extract in the oversight of diabetes mellitus.

In the present investigation, the tannin content was maximally present in hydro-ethanol (297 mg GAE/g) and followed by ethanol (277 mg GAE/g), methanol (202 mg GAE/g), chloroform (87 mg GAE/g), acetone (62 mg GAE/g) extract. Similar results were determined that the crude extract of polyherbal contains a higher amount of tannin in hydro-ethanolic extract versus other bioactive constituents (Monisha and Ragavan, 2015) [18]. Tannins are water-soluble phenol derivatives naturally synthesized and accumulated by higher plants as secondary metabolic products. Tannin is recited to be responsible for decreases in growth rate, feed intake, feed efficiency, protein digestibility, and net metabolizable energy, in experimental animals. Several tannin molecules have also been revealed to lessen the mutagenic activity of several mutagens. The anti-mutagenic and anti-carcinogenic potentials of tannins may be associated with their antioxidative property, which is significant in protecting cellular oxidative damage, subsuming lipid peroxidation. Their antimicrobial properties seemed to be associated with the hydrolysis of ester linkage between gallic acid and polyols hydrolyzed after ripening of many edible fruits. Tannins have also been reported to wield other physiological effects, such as reducing blood pressure, accelerating blood clotting, decreasing the serum lipid level, modulating immune responses, and producing liver necrosis (Chung *et al.*, 1998).

In the current investigation, the alkaloids content was maximum exhibited in methanol (59.51 mg AE/g) and followed by acetone (43.56 mg AE/g), hydro-ethanol (38.70 mg AE/g), ethanol (21.40 mg AE/g), and chloroform (19.78 mg AE/g) extracts. The leaf extract of *Salicornia virginica* contains a similar amount of alkaloids in the ethanolic extract as compared to other kinds of phytochemicals (Krishnaveni *et al*., 2016) [13]. Alkaloids are tremendously essential in medicine and comprise most of the important drugs. They have a noticeable physiological effect on animals (Edeoga *et al*., 2005) [6].

In this study, the phenol content was highest occurred in methanol (302 mg GAE/g) extract and leading by hydro-ethanol (232 mg GAE/g), ethanol (167 mg GAE/g), chloroform (102 mg GAE/g), and acetone (77 mg GAE/g) extracts. Similarly, methanolic extract of *Sphagneticola*

trilobata and *Adathoda vasica* shows a high quantity of phenolics in contrast to other phytochemicals (Kurapati *et al.*, 2018) [15]. The existence of phenol in the leaves of *Cichorium intybus* serves as antiseptic and lowers inflammation when held internally. These biologically active agents have an aggravation effect when used on the skin (Persinos *et al*., 1967) [21]. Cardiac glycosides have a strong and straight action on the heart, assisting in supporting its potency and rate of contraction when it is worsening (Persinos *et al*., 1967) [21].

5. Conclusion

The current study shows that methanolic extract of *Cichorium intybus* is enriched with essential nutrients. Qualitative phytochemical analysis showed that it is ample in phytochemicals namely alkaloids, flavonoids, saponin, tannin, phenol, proteins, carbohydrates, glycosides particularly found in the hydro-ethanol and methanol extracts than other extracts.In general, extraction yield augmented with increasing water content in ethanol, methanol, and hydro-ethanol system. This may be due to the combination of organic solvent and water that promotes the extraction of entire compounds that were soluble in both water and organic solvents. Quantitative analysis exhibited that methanolic extract contains maximum amounts of phytochemicals than the other types of the extracts such as hydro-ethanol, ethanol, chloroform, and acetone. From these investigations, it may be concluded that the methanolic extract of *Cichorium intybus* acts as the potential source of phytochemicals which may be used as conventional medicine for averting many diseases.

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