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Original Article

Development of Quality Standards and Phytochemical Investigation of *Cichorium intybus* L. seeds.

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ABSTRACT

Cichorium intybus Linn (Asteraceae) is a bushy perennial herb with blue, lavender, or occasionally white flowers. Chicory contain mainly volatile oils, alpha-amyrin, taraxerone, baurenyl acetate and beta-sitosterol, with the majority of the toxic components. In addition, the leaves of chicory may also be used as compresses to be applied externally to ease skin inflammations and swellings. As a mild diuretic, it increases the elimination of fluid from the body, leading to its use as a treatment for rheumatism and gout. The root and the leaves are appetizer, cholagogue, diuretic, hypoglycemic, laxative and tonic. The present study was designed to establish quality standards of *C. intybus*. Different Physiochemical Parameter like extractive values, ash values, were determine. Preliminary phytochemical screening was carried out to detect different phytoconstituents. Preliminary phytochemical screening of the extracts in different solvent revealed the presence of carbohydrates, phenolic compounds, flavonoids, alkaloids and proteins. Heavy metals were determined and found within acceptable limits. Pesticides residues and aflatoxins were also determined but not found in the tested samples. The physicochemical and phytochemical standards which are outcome of this research may be utilized as substantial data for identification and standardization of *C. intybus* seeds.

Keywords: *Cichorium intybus*, Pesticide, Aflatoxins, Phytochemical, Heavy metal

INTRODUCTION

Cichorium intybus L. seed is a member of the family Asteraceae. It is an important medicinal herb and used in Ayurveda, Unani and Siddha systems of medicine for diseases of hepatobiliary system and renal system. Recent studies have found some of the important constituents in chicory such as caffeic acid derivatives, flavonoids, inulin, and polyphe-nol in *C. intybus* L. (Maliakel et al.2008) Chicory is a widespread weed with antibacterial effect. Its habitants are road sides, railroads and waste grounds, flowering period lasts from June to October. Leaves of the plant contain salts such as sulphates and phosphates of sodium, magnesium and potassium as well as potassium nitrate. It also contains a bitter glycoside named cichorine. In traditional medicine, all parts of the plant specially root and leaves are used as diuretic, laxative, antibilious, antipyretic, blood purification and strengthen of the stomach. It is also used as an appetizer as well as in the treatment of hepatic failure, jaundice, intermittent fever and mild states of chronic skin diseases (Suzuki et al .1995) .The sesquiterpene lactones like lactucin and lactucopicrin were used for antibacterial and antimalarial activity(Rai et al.1996), antifungal activity(Kocsis.2003,Ghaderi et al.2012). Chichory also has antibacterial and nematicidal effect (kirtikar et al. 1997). However few scientific

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reports are available related to its Pharmacognostical and Phytochemical analysis. Therefore, this study was designed to establish quality standards of the drug.

MATERIALS & METHODS

Procurement of plant material: *Cichorium intybus* L seeds were procured from Yucca Enterprises, Bombay and samples were identified by taxonomist. The voucher specimen was deposited in Pharmacognosy and Phytochemistry Research Laboratory, Vivek College of Technical Education, Bijnor for further reference. The voucher no was NISCAIR/RHMD/Consult/-12/1781/81.

Macroscopical and microscopical evaluation: The plant material was subjected to macroscopical and microscopical evaluation. The seeds of *C. intybus* were observed carefully and preliminary observations were recorded. The seeds were powdered with the help of grinder and stained with different staining reagent to ascertain the presence of particular type of microscopical characters.

Physicochemical standardization:

Determination of extractive value: It is the amount of soluble constituents extracted with different solvents from a given amount of medicinal plant material. **(Harborne1992; Mukherjee2002)**

Cold Extraction: The air-dried coarse drug powder (10 gm) was macerated with solvent (Petroleum ether, chloroform, methanol and water) of volume 100 mL in a closed flask for 24 hours, shaking frequently during six hours and allowing standing for 24 hours. It is filtered rapidly, taking precaution against loss of solvent, the filtrate evaporated to dryness in a tarred flat bottom dish and dried at 105ºC, to constant weight and percentage yield was calculated.

Hot Extraction: The powdered material of the drug (10 gm) was packed in a Soxhlet apparatus separately for each solvent like petroleum ether, chloroform, methanol and water. Each extract was evaporated to dryness and constant extractive value was recorded.

Successive Extraction: The dried and coarsely powdered material (10 gm) was subjected to successive extraction in a Soxhlet apparatus with different solvents like petroleum ether, chloroform and methanol. The extracts were evaporated to dryness and their constant extractive values were recorded.

Determination of ash values:

Ash values: This constraint can be used for the determination of inorganic materials, such as carbonates, silicates, oxalates and phosphates. Heating causes the loss of organic material in the form of CO_2 leaving behind the inorganic components. Ash value is an important characteristic of a drug and with the help of this parameter we can detect the extent of adulteration as well as establish the quality and purity of the drug. There is a considerable difference in the ash values of different drugs but mostly the difference varies within narrow limits in case of the same drug. The acid insoluble ash consists mainly of silica and high acid insoluble ash thereby indicating the contamination with earthly materials. The water-soluble ash is used to estimate the amount of inorganic elements

Determination of total ash values: Ignition of medicinal plant material yields total ash constituting both physiological (from the plant tissue) and non-physiological (extraneous matter adhering to the plant) ash. The ground drug was incinerated in a silica crucible at a temperature not exceeding 450 ºC until free from carbon. It was then cooled and weighed to get the total ash content.

Determination of Acid insoluble ash values: Acid insoluble ash represents sand and siliceous earth. Ash is boiled with 25 mL dilute HCl (6N) for five minutes. The insoluble matter collected on an ash less filter paper, washed with hot water and ignited at a temperature not exceeding 450 ºC to a constant weight.

Determination of Water-soluble ash values: Ash was dissolved in distilled water and the insoluble part collected on an ash less filter paper and ignited at 450ºC to constant weight. By subtracting the weight of insoluble part from that of the ash, the weight of soluble part of ash was obtained.

Florescence analysis: Many herbs fluorescence when cut surface or powder is exposed to UV light and this can help in their identification method. The fluorescence character of the plant powders (40 mesh) was studied both in daylight and UV light (255 and 366 nm) and after treatment with different reagents like sodium hydroxide, picric acid, acetic acid, hydrochloric acid, nitric acid, iodine, ferric chloride etc.**(Chase1949; Kokoshi1958)**

Phytochemical screening: The Petroleum ether extract, Chloroform extract, Methanolic extract, aqueous extract of the plant material were subjected to preliminary phytochemical investigation for the detection of secondary

metabolites. (**Mukherjee2002).** The screening was performed for Alkaloids, Carbohydrates, Phenolic compounds, Flavonoids, Protein, Saponins, mucilage, resins and lipids or fats etc.

Heavy Metal Residues: Residues of heavy metals (Cd, Pb, As and Hg) in the extracts were determined according to the American Organization of Analytical Chemists (AOAC) official method of analysis (**Anonymous 2002)** (**Anonymous 2003)** Lead, cadmium, arsenic and mercury are the most common toxic metals that have become a matter of concern due to the reports of their contamination in various herbal preparations and herbal ingredients. (**Mukherjee2002).** Lead is known to cause neurological disorders, anemia, kidney damage, miscarriage, lower sperm count and hepatotoxicity in higher concentration. Acute or chronic exposure of cadmium causes respiratory distress, lung and breast cancers, hemorrhagic injuries, anemia and cardiovascular disorders. **Kokosni(1958).**Arsenic is reported to cause hypertension, peripheral arteriosclerosis, skin diseases and neurotoxicity. Determination of heavy metals (Cadmium, Lead, Arsenic and Mercury) analysis was carried out in the extracts of *C.intybus* on Atomic Absorption Spectrophotometer (AAS). All necessary precautions were taken to avoid any possible contamination of the sample as per the AOAC guidelines. (**Anonymous 2002)** (**Anonymous 2003).**

Pesticide Residues: Pesticides (organochlorines, organophosphates and pyrethroids) residues in the extracts were determined by GC-MS according to AOAC guidelines. Pesticides are the toxic substances; the drugs should be free from these substances. (**Anonymous 2002)** (**Anonymous 2003).**

Aflatoxin Analysis: Aflatoxins were analyses in extracts of *C.intybus* by HPLC method as described by AOAC guidelines (**Anonymous 2002)** (**Anonymous 2003)** Mycotoxins are secondary metabolites produced by fungi that develop naturally in food products. These toxins may lead to a great variety of toxic effects in vertebrates, including humans. Toxigenic fungi may contaminate herbal products at different phases of production and processing, mainly in favorable humidity and temperature conditions. Many mycotoxins also have significant chemical stability, which enables their persistence in products even after the removal of the fungi by means of the usual manufacturing and packaging processes. The most common toxigenic fungi found in plants include species from the genera *Aspergillus* and *Fusarium*, mainly *Aspergillus flavus*, *Aspergillus parasiticus* and *Fusarium verticillioides*. Aspergillus species produce aflatoxins B_1, B_2, G_1 and G_2 which are considered to be involved in the etiology of human liver cancer. **(Samson etal 2001).**

RESULTS AND DISCUSSION

The macroscopical study of the *C. intybus* L seeds was done. The seeds were pale brown to grey in colour, 3 mm in size, oval in shape with flat surface, strong odour & bitter taste (Table-1, Fig-1). The microscopic examination of powdered material was performed to detect and established various identifying microscopic characters which will be help full in differentiation of the substitute of the drug supplied in the form of dried powder. The photomicrographs of the identifying features of the plant material are shown in (Fig 2-5).

The trichomes are unicellular, thick and short curve at the base non-lignified. The fibre are lignified which are present in the groups of two fibres. Some pitted vessels are also present in the drug. The values of the physical constant like ash values, extractive value were determined. The various physiochemical parameters like ash values and extractive value were also determined shown in (Table-2). Fluorescence nature of the powder Cichory drug in different solvent extracts with different chemicals were analyze using longer light wavelength (366 nm) and shorter light wavelength (254 nm) are reported in (Table 3).Preliminary qualitative phytochemical screening shown that presence of alkaloids, carbohydrates, flavonoids and Phenolic compound and amino acid , Presence or absence of particular types of phytoconstituents in the plant of the interest may be helpful, partly in the development of analytical profile and in the differentiation of contravention plants. The results of the studies performed are tabulated in (Table- 4). Determination of heavy metals (Cadmium, Lead, Arsenic and Mercury) analysis was carried out in the extracts of *C. intybus* on Atomic Absorption Spectrophotometer (AAS). All necessary precautions were taken to avoid any possible contamination of the sample as per the AOAC guidelines. Cd was found in *C. intybus* (0.20 ± 0.02mg/kg) which was below the permissible limit of 0.3 mg/kg as prescribed by WHO. Pb ranged from 0.16 ± 0.06 mg/kg to 0.43 ± 0.05mg/kg in drug samples and was far below the permissible limit of 10 mg/kg as prescribed by WHO. Hg in drug samples was found to be below the permissible limit. As and Hg was detected in drug sample. Both metals were found to be within permissible limits of 0.5 ppm and 1.0 ppm respectively (Table-5 and Figure-6). Determination of pesticide residue was carried out in extracts of *C. intybus* by standard methods as described in

AOAC guidelines. Total 30 pesticides were tested in all the samples, none of the pesticides was found in samples of the extracts (Table-6). Mycotoxins are secondary metabolites produced by fungi that develop naturally in food products. These toxins may lead to a great variety of toxic effects in vertebrates, including human .Toxigenic fungi may contaminate herbal products at different phases of production and processing, mainly in favorable humidity and temperature conditions. Many mycotoxins also have significant chemical stability, which enables their persistence in products even after the removal of the fungi by means of the usual manufacturing and packaging processes.

The most common toxigenic fungi found in plants include species from the genera *Aspergillus* and *Fusarium*, mainly *Aspergillus flavus*, *Aspergillus parasiticus* and *Fusarium verticillioides* Aspergillus species produce aflatoxins B_1, B_2, G_1 and G_2, which are considered to be involved in the etiology of human liver cancer. Aflatoxins B_1, B_2, G_1 and G_2 were determined in the extract of *C. intybus* No aflatoxin was detected in any sample of the extract. (Table 7). The outcome of this study might prove beneficial in herbal industries for identification, purification and standardization of *C. intybus* L. seeds extract

CONCLUSION

The generated data of the present investigation might be used to establish its quality and purity and may be utilised to develop pharmacopoeial monograph of *C. intybus* L. seeds. The outcome of this study might prove beneficial in herbal industries for identification, purification and standardization of *C. intybus* L. seeds extracts.

Table: 1 Macroscopical characters of *Cichorium intybus*

S. No	Description of the macroscopic structure	Observation
1	External colour	Pale brown to grey
2	Size	3 mm
3	Shape	Oval
4	Surface	Flat
5	Odour	Strong
6	Taste	Bitter & Spicy

Figure1: Seeds of *Cichorium intybus* L.

Table 2: Summary of results of physicochemical evaluation of drug

S. No	Parameters		% w/w (Mean ± SEM)
1	**Ash values**	Total Ash	28.88 ± 2.76
		Acid insoluble Ash	2.17 ± 0.67
		Water soluble Ash	2.75 ± 0.76
2	**Successive extraction**	Petroleum ether	2.056 ± 0.56
		Chloroform	1.26 ± 0.084
		Methanol	5.13 ± 0.87
		Water: alcohol (50:50)	3.93 ± 0.45
		Water	1.97 ± 0.053

Fig:2- Magnified view of trichomes

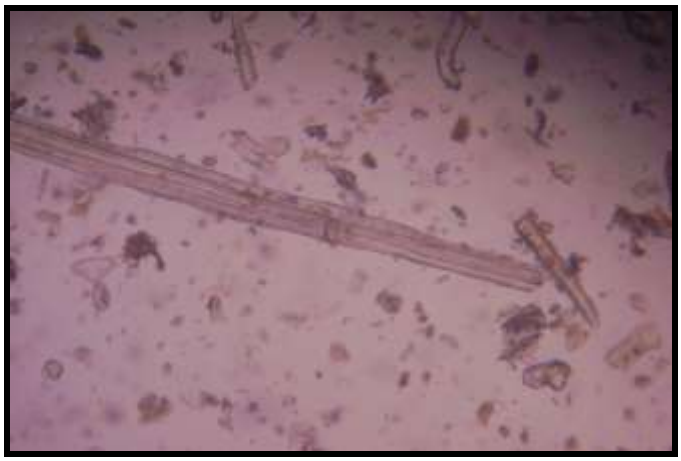

Fig:3- Magnified view of fibres and trichomes

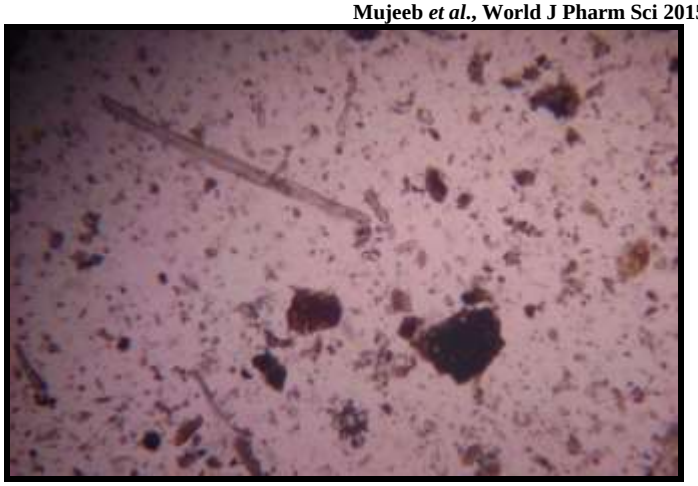

Fig:4- fibres with trichomes

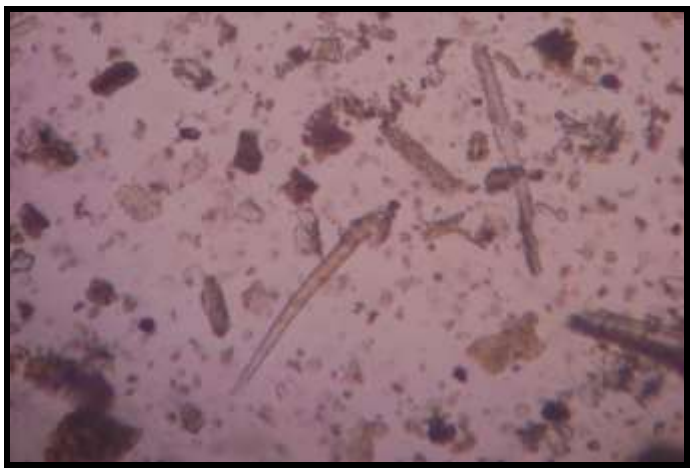

Fig:5- Trichomes with pitted vessels

Table: 3 - Effect of different chemical reagents on the fluorescence behaviour of crude drug powder

Treatment	Day light	UV light 254 nm	UV light 366 nm
Powder as such	Light brown	Dark brown	Brown
Powder treated with distilled water	Light brown	brown	Brown
Powder treated with 1N NaOH in water	Greenish brown	Greenish black	Brown
Powder treated with HNO_3	Light brown	Dark green	Dark violet
Powder treated with H_2SO_4	Green	Black	Blue
Powder treated with iodine	Green	Blue	Brown
Powder treated with conc. HCl	Dark green	Radish brown	Greenish black
Powder treated with ammonia	Light green	Dark green	Greenish
Powder treated with ferric chloride	Green	Radish black	Greenish brown
Powder treated with Iodine	Dark brown	Brown	Brown
Powder treated with Glacial acetic acid	Yellow	Dark yellow	Yellow
Powder treated with Picric acid	Dark yellow	Yellow	Dark yellow
Powder treated with Petroleum ether	Dark green	Green	Dark green
Powder treated with Chloroform	Dark green	Dark green	Dark green

Table: 4- Results of Phytochemical screening

Phytoconstituents	Extracts			
	Petroleum ether	**Chloroform**	**Alcoholic**	**Aqueous**
Alkaloids	-	+	+	+
Carbohydrates	-	-	+	+
Phenolic compounds	-	+	+	+
Flavonoid	-	+	+	+
Proteins and amino- acids	-	-	+	+
Saponins	-	-	-	-
Mucilage	-	-	+	+
Resins	-	-	-	-
Lipids / Fats	+	-	-	-

(-: Absent, +: Present)

Table 5: Determination of heavy metal residues

S. No	Heavy Metals	Concentration
1	Cadmium (Cd)	0.20± 0.05
2	Lead (Pb)	0.43 ± 0.05
3	Arsenic (As)	0.16 ± 0.07
4	Mercury (Hg)	0.06 ± 0.02

Limit described by the WHO (Lead- 10 ppm; Cadmium-0.30 ppm; Mercury-0.50 ppm; Arsenic-3.0 ppm)

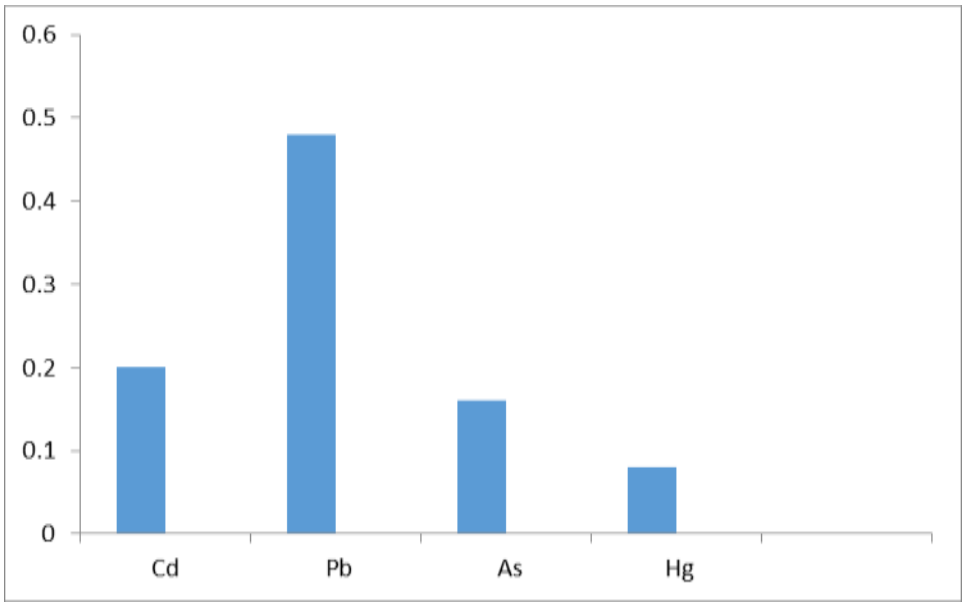

Fig-6: Heavy metal residue for *Cichorium intybus*

Table 6: Determination of pesticide residue

S. No	Pesticide	Test method	Results	MDL
1	α -BHC	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
2	β-BHC	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
3	γ-BHC(Lindanee)	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
4	δ-BHC	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
5	Heptachlor	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
6	Heptachlor_Epoxide	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
7	α -Chlordane	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
8	α -Endoulfan	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
9	β -Chlordane	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
10	Endrin	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
11	Total DDE	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
12	Total DDD	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
13	Total DDT	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
14	β -Endoulfan	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
15	Endrin_Aldehyde	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
16	Alachlor	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
17	Butachlor	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
18	Monochlorphos	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
19	Phorate	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
20	Mevinphos	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
21	Dimethoate	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
22	Malathion	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
23	Methyl-parathion	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
24	Chlorpyrifos	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
25	Ethion	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
26	Atrazine	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
27	Simazine	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
28	Diazinone	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
29	Phosphamidon	AOAC970.52/EPA525.5	Not detected	0.01mg/kg
30	Fenitrothion	AOAC970.52/EPA525.5	Not detected	0.01mg/kg

Table 7: Determination of aflatoxin residues

S. No	Test parameter	Test method	Results	MDL
1	AflatoxinB1	AOAC 990.332	Not detected	1.0µg/kg
2	AflatoxinB2	AOAC 990.33	Not detected	1.0µg/kg
3	AflatoxinG1	AOAC 990.33	Not detected	1.0µg/kg
4	AflatoxinG2	AOAC 990.33	Not detected	1.0µg/kg

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