International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Unveiling the therapeutic potential of *Cichorium intybus* callus: a phytochemical study

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Abstract

The present study focuses on the development and phytochemical investigation of Chicory callus culture. The Chicory root callus showed presence of glycosides, terpenoids and even toxins. The callus cultures are known to exhibit the phytoconstituents that are not present in mother plants naturally. The culture showed the presence of many compounds like 3beta,6beta-Dihydroxynortropane, Saxitoxin, E-64, Isomasticadienonalic acid, Onchidal, etc which are not present in Chicory plant naturally. Additionally, the callus extract revealed presence of several phytoconstituents like cis-1,3,4,6,7,11b-Hexahydro-9- methoxy-2H-benzo[a]quinolizine-3-carboxylicacid, 3,5,9-Trihydroxyergost-7-en-6-one, 4,4-Disubstituted cyclohexenone whose pharmacological activity is not known yet.

Keywords: Chicory, Cichorium intybus, Callus, Phytochemical evaluation

Full length article **Corresponding Author*, e-mail: wadekar_p@yahoo.co.in

1. Introduction

Many people worldwide continue to depend on traditional medicine systems that utilize drugs derived from natural sources. This reliance on natural remedies reflects their long-standing effectiveness and cultural importance, passed down through generations within various communities. Natural origin drugs still continue to be the focus of pharmaceutical research because the drug discovery methods have failed to provide lead compounds for treatment of various disease [1]. Chicory (*Cichorium intybus* Linn belonging to Asteraceae family) is a perennial plant found across different parts of the world. It is used as livestock forage, in traditional remedies, and as a dietary vegetable. Originating in Egypt around 4000 BC, chicory has gained increased attention due to its nutritional and medicinal value. It's notably used for caffeine-free coffee substitutes, coffee additives, and as a vegetable and probiotic element, especially prominent in the Mediterranean region [2]. The bitter taste of Chicory, has garnered growing attention due to its nutritional richness and medicinal properties. It contains secondary metabolites like terpenoids and polyphenols, particularly phenolic acids.

The phenolic acids exhibit antioxidant, anti-inflammatory effects, and potential insulin-like activity properties [3]. Additionally, chicory is recognized for its ability to demonstrate anticarcinogenic, antiviral, antibacterial, antimutagenic, antifungal, anthelmintic, immune-stimulating, and antihepatotoxic effects [4]. The anti-hepatotoxic activity of chicory root callus is already reported [5]. But no detailed investigation regarding the phytochemical analysis of the chicory root callus was performed. The present study focuses on development and phytochemical analysis of Chicory root callus.

2. Materials and methods

2.1. Preparation of Callus

The Chicory seeds were collected from Neeraj traders Jhansi, Indian and were sown in the month of July. The plants bloomed in November. The plant was authenticated form Botanical Survey of India, Pune. The root explants were surface sterilized. Murashige and Skoog agar media with different concentrations of 2,4-Dichlorophenoxy acetic and kinetin was used for callus induction.

The explants were incubated at 25± 2ºC under 16 hr photo period of 2000 lux with white light. The relative humidity was maintained at 60-70% during incubation at Seem Biotech Pvt Ltd, Warananagar, India. Sub-culturing was carried out every six weeks. After 6 months, the callus cultures were weighed and callus index was calculated.

2.2. Evaluation parameters for callus

Callus index is a parameter used to access the growth rate of the tissue [6]. Callus index was calculated using following formula:

$$\textbf{Callus inex} = \frac{\mathbf{n} * \mathbf{G}}{\mathbf{N}} * \mathbf{100}$$

n= Total number of callused explants
G= Average weight of callus rating on explant
N= Total number of cultured explants

2.3. Extraction of Callus

The callus culture obtained was dried at 50±5°C in hot air oven and was subjected to ethanolic extraction using Soxhlet apparatus and subsequently dried in vacuum dryer.

2.4. Phytochemical analysis of Callus extract

The dried callus extract was sufficiently diluted in ethanol. The detailed phytochemical investigation was performed by liquid chromatography mass spectrometry (LCMS).

3. Results and Discussions

3.1. Development of callus

The callus for Chicory root was developed by using 2 ml of 2,4- Dichlorophenoxy acetic acid solution (1mg/ml) in 1 Liter of Murashige Scoog medium. The callus index was 28.54 indicating satisfactory callus growth.

3.2. Callus extract

The percentage practical yield for callus extract was 9.32%.

3.3. Phytochemical analysis of callus extract

The chromatograms for positively charge compounds and negatively charged compounds are shown in figure 2 and 3. The callus extract showed presence of glycosides (2",4"-Diacetylafzelin, 2,3-Butanediol glucoside, Norrubrofusarin 6-beta- gentiobioside, 3-Methylellagic acid 8- rhamnoside, Medicagenic acid 3-O-beta-D- glucoside, Cnidimol 7-glucoside, 2a-Hydroxygypsogenin 3-O-b- D-glucoside, Ponasteroside A) terpenoids (Oryzalexin A, Isomasticadienonalic acid, Ganosporelactone A, Medicagenic acid 3-O-beta-D- glucoside, (3beta,19alpha)-3,19,23,24- Tetrahydroxy-12-oleanen-28- oic acid, Valdiate) and even toxins (Saxitoxin, Miserotoxin) . The callus cultures are known to exhibit the phytoconstituents that are not present in mother plants naturally [59].

The culture showed the presence of many compounds like 3beta,6beta- Dihydroxynortropane, Saxitoxin, E-64, Isomasticadienonalic acid, Onchidal, etc which are not present in Chicory plant naturally. We also got many compounds like cis-1,3,4,6,7,11b-Hexahydro-9- methoxy-2H- benzo[a]quinolizine-3- carboxylic acid, 3,5,9-Trihydroxyergost-7-en-6- one, 4,4-Disubstituted cyclohexenone whose pharmacological activity in not known yet. We can further go for isolation of these compounds to check the pharmacological potential.

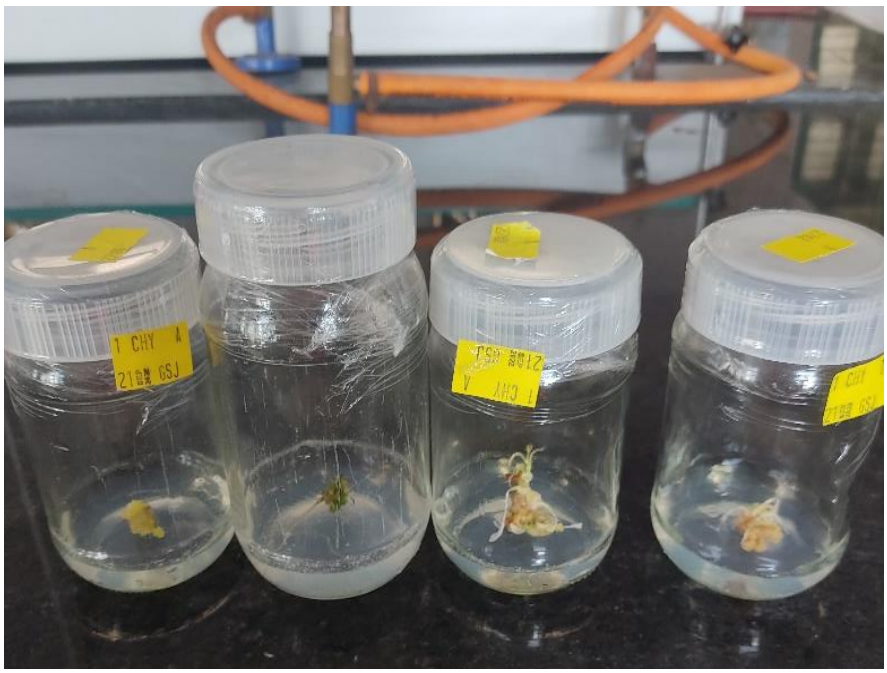

Figure 1. Chicory root callus

Table 1: LCMS instrumentation specifications for detection of positively charged compounds

Sample Name	CC	**Position**	P2-E3	**Instrument Name**	QTOF	**User Name**	
Inj Vol	3	**Inj Position**		**Sample Type**	Sample	**IRM Calibration Status**	Success
Data Filename	CC.d	**ACQ Method**	Metabolite ESI_+VE_M	**Comment**		**Acquired Time**	4/6/2023 9:21:49 PM

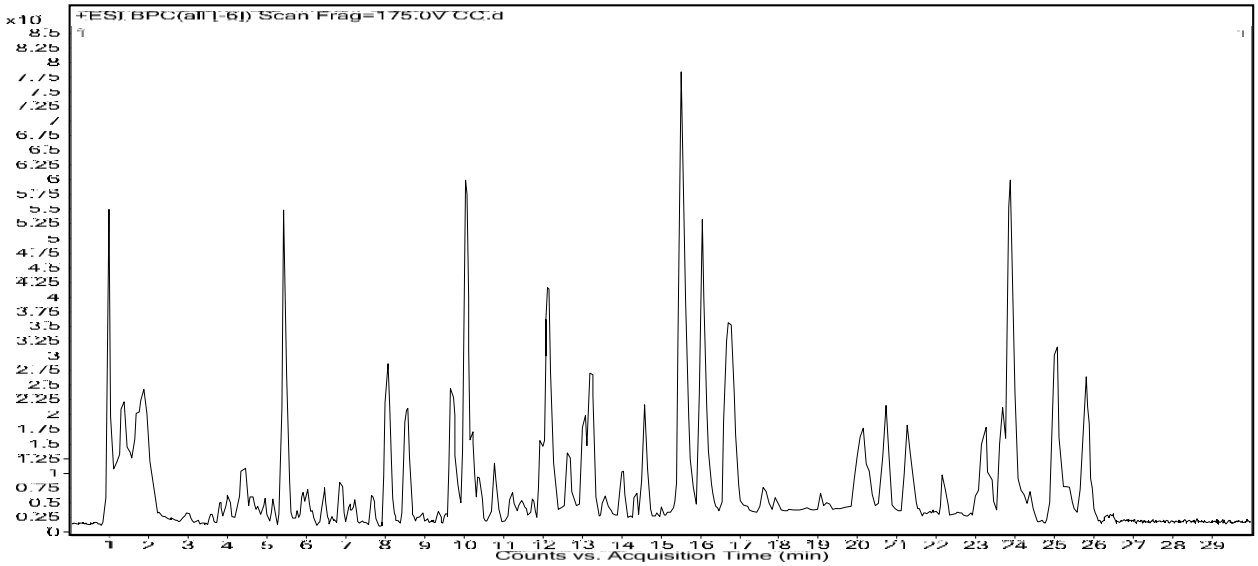

Figure 2. Chromatogram for positively charged compounds of Chicory callus extract

Table 2: LCMS instrumentation specifications for detection of negatively charged compounds

Sample Name	CC	**Position**	P2-E3	**Instrument Name**	QTOF	**User Name**	
Inj Vol	3	**Inj Position**		**Sample Type**	Sample	**IRM Calibration Status**	Success
Data Filename	CC- ve.d	**ACQ Method**	Metabolite ESI_-VE_M	**Comment**		**Acquired Time**	4/7/2023 6:14:04 AM

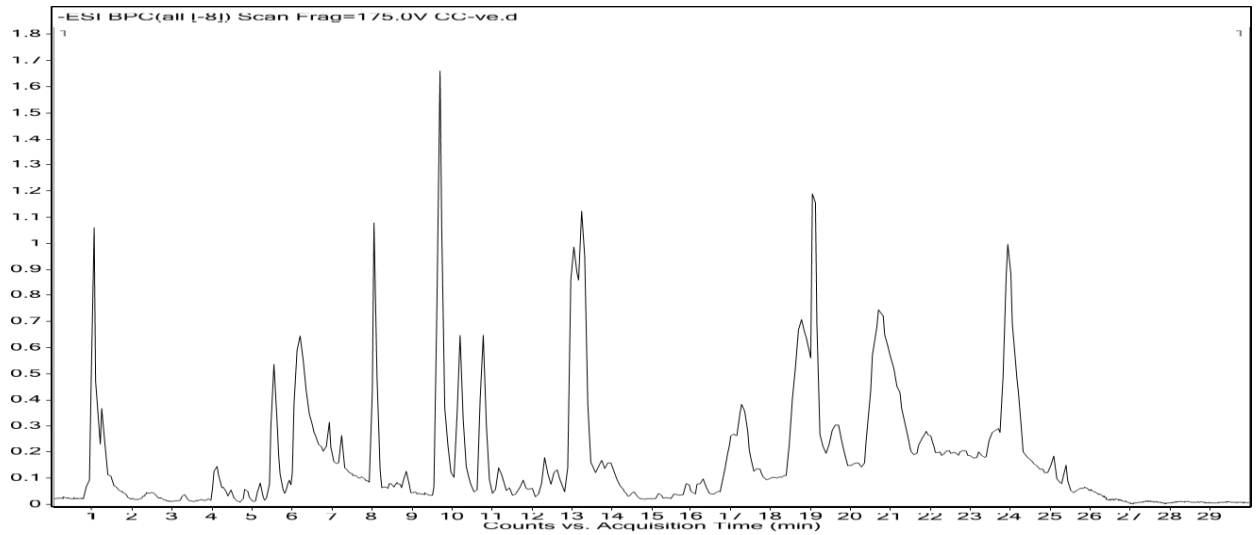

Figure 3. Chromatogram for negatively charged compounds of Chicory callus extract

Table 1. Phytochemical analysis of Callus

Sr. no	Name	Pharmacological activity
1	3beta,6beta- Dihydroxynortropane	Antidiabetic [7-9]
2	Anapheline	Anti-thrombotic effects [10]
3	Anthranilic acid	Prevents SEIZURES or reduce their severity [11]
4	Saxitoxin	Neurotoxin [12-13]
5	2,3-Butanediol glucoside	Pharmacological activity not known yet
6	Miserotoxin	Phytotoxin [14]
7	E-64	Cysteine protease inhibitor [15] In treatment of Leishmania infection [16]
8	PAB / 4-Aminobenzoic acid	Scleroderma, dermatomyositis and Peyronie's disease [17]
9	(alphaS, betaS)-alpha-Ethyl-alpha-(4-methoxyphenyl)-beta-phenyl-2-pyridineethanol	Pharmacological activity not known
10	2-Hydroxy-6-oxo-octa-2,4- dienoate	Activity not known
11	(E)-2-Methyl-2-buten-1-ol O- beta-D-Glucopyranoside	Activity not known
12	7-Hydroxy-6-(methoxyacetyl)-2,2-dimethyl-2H-1-benzopyran	Activity not known
13	Isomasticadienonalic acid	Triterpenoid anti-inflammatory activity [18-19]
14	Chrysoidine free base	In the treatment of prion disease [20]
15	Dexchlorpheniramine	Anti-histamine, anti-allergic activity [21]
16	Dihydrocapsaicin	Cancer prevention, cardiovascular and gastrointestinal benefits, antiarthritic pain control, anti-inflammatory and antioxidant activities, and weight loss properties [22]
17	cis-1,3,4,6,7,11b-Hexahydro-9-methoxy-2H- benzo [a] quinolizine -3- carboxylic acid	Activity not known
18	Onchidal	Neurotoxin [23]
19	3,5,9-Trihydroxyergost-7-en-6- one	Activity not known
20	3-Methoxy-D-homoestra-1,3,5(10),8-tetraen-17a-one	Activity not known
21	4,4-Disubstituted cyclohexenone	Activity not known

22	Anapheline	Anti-platelet and anti-thrombotic effect [24]
23	Furfuryl octanoate	Flavoring Agents [25]
24	Lophocerine	In the treatment of cancer [26-27]
25	C16 Sphinganine	Cell growth, Differentiation, Apoptosis [28-29]
26	Yucalexin P8	Cyclohexenones Activity not known
27	7-Oxatyphasterol	Steroid Activity not known
28	5,6-Epoxiergosta-8,22-diene- 3,7-diol	Steroid Activity not known
29	Methyl 3-(2,3-dihydroxy-3-methylbutyl)-4- hydroxybenzoate	Antifungal activity [30-31]
30	Jasmine ketolactone	Activity not known
31	2-(3-Phenylpropyl) tetrahydrofuran	Flavouring agent [32]
32	Mitoxantrone	Antineoplastic agent
33	Polyporusterone F	Useful in the treatment of leukemia [33]
34	Sphinganine	Cell growth, Differentiation, Apoptosis [28-29]
35	Pyruvophenone	Flvouring agent [34]
36	(5alpha,8beta,9beta)-5,9-Epoxy-3,6-megastigmadien-8- ol	Nutrient [35]
37	(13R,14R)-8-Labdene- 13,14,15-triol	Anticancer [36]
38	Norethindrone	Syntheitc origin.
39	Teasterone	For treatment of hypogonadism [37]
40	17-Methyl-18-norandrosta-4,13(17)-dien-3-one	Steroid Activity not know yet
41	Diplodiatoxin	Fungal toxin [38]
42	17beta-Hydroxy-2-methylandrost-1,4-dien-3-one	A steroid Pharmacological activity not known
43	Norrubrofusarin 6-beta- gentiobioside	Antioxidant [39]
44	Isomasticadienonalic acid	Antiinflammatory [40]
45	Phthalic acid Mono-2- ethylhexyl Ester	Synthetic origin.
46	Alpha-CEHC	Antioxidant [41-43]
47	19-Noretiocholanolone	Androgenic activity [44]
48	Smilagenone	For treatment of neurodegeneration diseases [45]
49	(25R)-5beta-spirostan-3beta- ol	Antineoplastic [46]
50	3-Ketosphinganine	In treatment of cancer [47]
51	Oryzalexin A	Antimicrobial [48-49]
52	Plaunotol	Antibacterial, Antiulcer drug [50-51]
53	Ganosporelactone B	In treatment of cancer [52]
54	Euphornin	In treatment of cancer [53-54]
55	Pyropheophorbide a	A photosensitizer, anticancer and antiviral activities [55-57]
56	2-Caffeoylisocitrate	Activity not known yet
57	Ganosporelactone A	Anti-HIV-1 protease, anti-tumor, and anti-complement activities [58]

4. Conclusions

The chicory callus culture exhibited the presence of glycosides, terpenoids, and flavonoids. This culture presents an alternative method to extract phytoconstituents without harming the plant, achieving results in a shorter time frame. The study explored the pharmacological potential of chicory root callus in addressing various conditions such as diabetes, atherosclerosis, seizure severity, Leishmania infection, scleroderma, dermatomyositis, Peyronie's disease, and as an anti-inflammatory agent, depending on the detailed phytochemical analysis. It has also opened a door for development of new drug molecules.

5. Declarations

Acknowledgements

The authors gratefully thank to Seema Biotech, Pvt Ltd, Warananagar, India; Indian Institute of Technology, Bombay; Shivaji university, Kolhapur; Appasaheb Birnale College of Pharmacy, Sangli, Indian and Rajarambapu College of Pharmacy, Kasegaon, Sangli, India for materials and technical assistance.

Conflict of Interest

The authors of this research article have no conflicts of interest to disclose.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Ethics Statement

Not applicable

Informed Consent

Not applicable

Data Availability

The data for fig 2-3 and Table no 1-3 is available on request.

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