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Evaluation of Antimycotic potential and qualitative phytochemical analysis of seed, root and leaf extract of *Cichorium intybus* L

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Abstract
Cichorium intybus has long being traditionally used for medicinal purposes. Present study has been done to check the antibacterial potential of various solvent extract of root, seed and leaf of plant against certain selected fungal species i.e, *Aspergillus niger*, *Alternaria alternata*, *Penicillium notatum*, *Trichoderma viride*. The qualitative phytochemical analysis has also been performed to determine the chemical composition of the crude extract of seed, root and leaves. Phytochemical analysis of various extracts of root, seed and leaves shows presence of active phytochemical components such as alkaloids, flavonoids, saponins, tannins, steroids. The results of agar well diffusion assay deduced that extracts of various plant parts of *C. intybus* shows a moderate amount of effect against the growth of *Alternaria alternata* and *Trichoderma viride* fungi. But the antifungal potential of different some extracts like spirit, chloroform, water showed considerably higher amount of antifungal activity against *Alternaria alternata* and *Penicillium notatum.*

Keywords: Antimycotic activity, Phytochemistry, agar well diffusion, mic

1. Introduction
Cichorium is a genus of plants within the sunflower family. The genus includes two cultivated species commonly known as chicory (*Cichorium intybus)* and endive (*Cichorium endivia*), and four wild species and several cultivars with major geographical range in Europe and Asia. It occurs throughout North West India in Punjab, Kashmir, Andhra Pradesh, Karnataka and Maharashtra and Rajasthan. In Rajasthan its distribution is all along the desert and Aravali range.

Common chicory (*Cichorium intybus*) is a bushy perennial herb around 1 m in height with a fleshy tap root of around 75 cm in length, with blue or lavender (or, rarely, white or pink) flowers [1, 2]. It is grown for its leaves, when it is known as leaf chicory, endive or witlo of. Other varieties are grown for their roots, which are used as a coffee substitute, similar to dandelion coffee. It is well known as a coffee substitute but is also widely used medicinally to treat various ailments ranging from wounds to diabetes.

Chicory, *Cichorium intybus*, the whole plant contains a number of medicinally important compounds such as inulin, esculin, volatile compounds, bitter sesquiterpene, lactones, coumarins, flavonoids and vitamins [3-5]. Ground roasted powder of the roots is mixed with coffee to impart rich flavor and to decrease caffeine content of the coffee formulation [6]. The plant is a good tonic, cooling and is useful in headache, throat inflammation, immune-stimulation, mutagenic, probiotic, hepatoprotective, antibacterial activity etc are the other pharmacological actions attributed to a wide range of phytochemical constituents present in this plant. The main chemical components of *C. intybus* are sesquiterpenes lactones, such as lactucin, 8- deoxylactucin, lactupicrin and 11b-dihydro-terpene derivatives [7]. Other chemical costituents found are coumarins such as cichoriin, umbelliferone, esculin, scopoletin and 6,7-dihydeoxycoumarin [8], vitamins [9], flavone derivatives like cichoric acid, chlorogenic acid, apigenin, quercitin [10], carbohydrates like glucose, fructose and inulin [11]. The function of the secondary metabolites of chicory could perhaps be connected with a passive defense of the plant against pathogens [12]. Pharmacological actions reveals that plant also possess anti-carcinogenic, hypoglycemic, hepatoprotective anti-ulcer potentials [13].

2. Material and Methods
2.1 Plant Material
Fresh samples of whole plant have been collected. Different plant parts were separated and they were allowed to get completely dried at room temperature under shade and then they were mashed into fine powder.

2.2 Preparation of Extract

About 50 gm of powdered material is taken and soaked in 150 ml in each solvent one after the other in sequential order- Pet Ether, Chloroform, Acetone, Spirit, Water for 2 weeks, shake well 2-3 times a day and then filtered. The filtrate then is allowed to dry or evaporate in incubator at 40 ºC. All the extracts were dissolved in DMSO, than they were stored in clean and sterile glass vials.

2.3 Bacterial Specimen

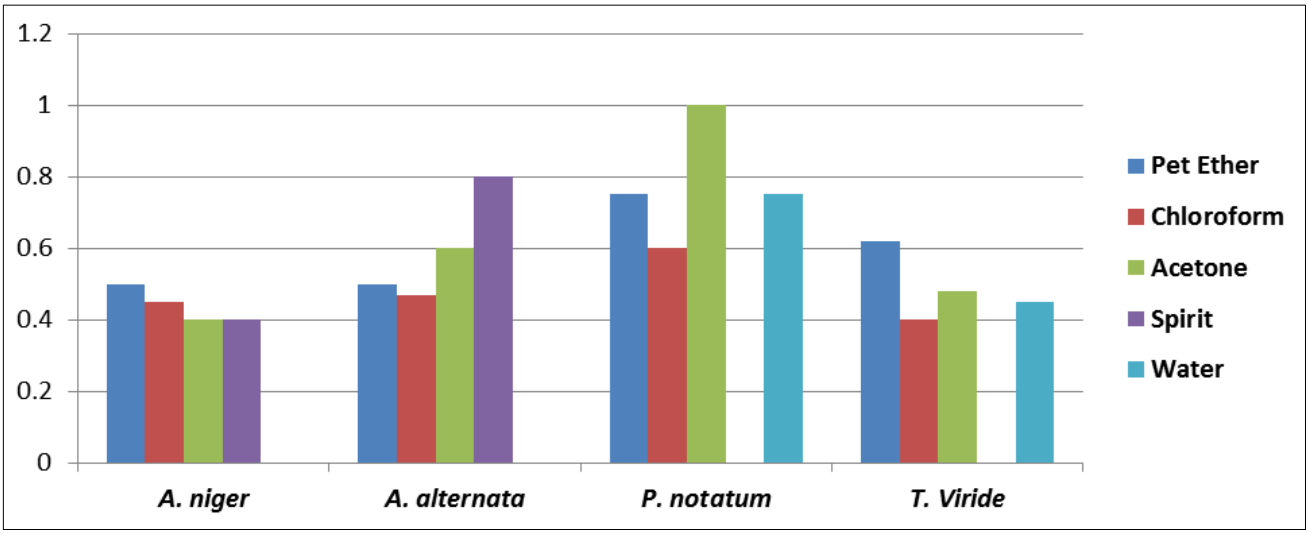
Three fungal strains *Aspergillus niger*, *Alternaria alternata*, *Penicillium notatum*, *Trichoderma viride* has been taken against which the antifungal activities of plant extract were tested. All the fungal strains were obtained from S.M.S. Medical College, Jaipur.

2.4 Antibacterial Assay

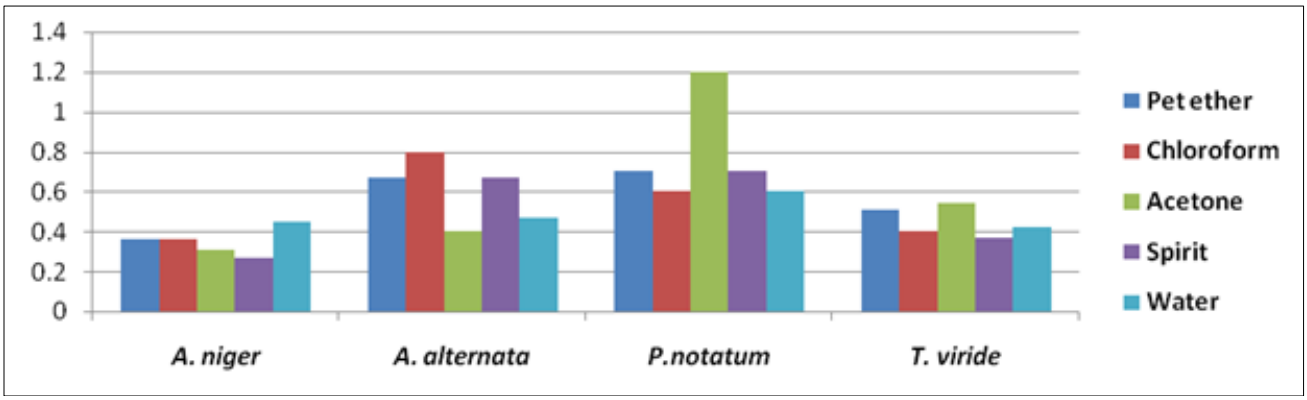
Antimycotic potential of different extracts of *C. intybus* was evaluated by using disk diffusion method via measuring the inhibition zone exhibited by various extracts.The different soluble solvent fractions were subjected for screening the antifungal potential against four different fungi strains. The antifungal bioassay was determined by Agar well diffusion method. The fungus culture was inoculated in potato dextrose agar media. Fungal specimens were spread over the solidified and cooled agar plates, then on the inoculated agar plates wells of 5mm were carved by using sterile borer. Extract/fractions were dissolved in 10% sterile dimethyl sulfoxide. The discs (6 mm in diameter) were impregnated with50 mg/mL concentration extract/fractions (60µL/disc) placed on the inoculated agar. The plates were then incubated at 40ºC. After 24 hrs the bacterial growth was checked and the antibacterial activity were measured as diameter of the zone of inhibition exhibited by different solvent extracts and compared with the zone of inhibition shown by the standard antibiotic.

Table 1: Determination of Zone of Inhibition and activity index of various solvent extracts of seed, root and leaf.

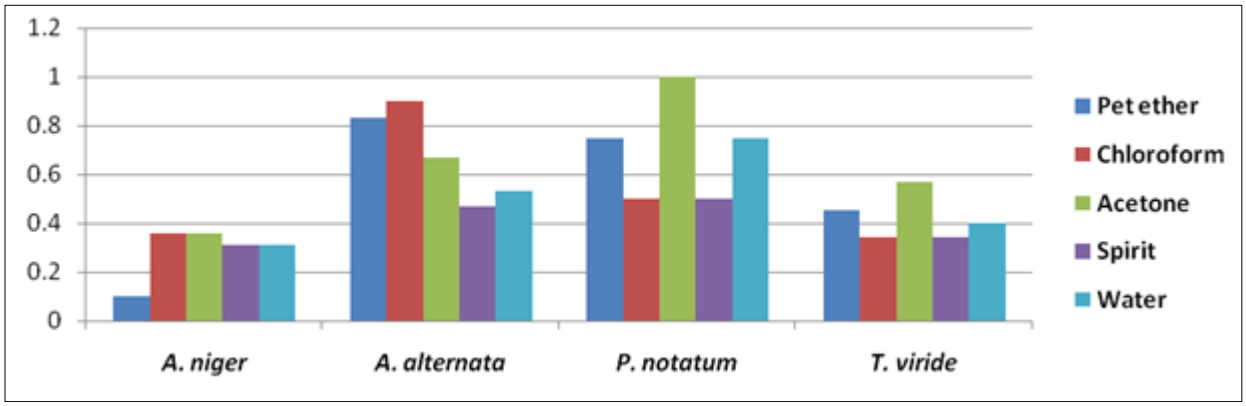
Fungus		Seed					Root					Leaf				
		P	C	A	S	W	P	C	A	S	W	P	C	A	S	W
A. niger	ZI	22	18	20	20	-	16	16	14	12	20	14	16	16	14	14
	AI	0.5	0.45	0.4	0.4	-	0.36	0.36	0.31	0.27	0.45	0.31	0.36	0.36	0.31	0.31
A. alternata	ZI	16	14	20	25	-	20	24	12	20	14	25	27	20	14	16
	AI	0.5	0.47	0.6	0.8	-	0.67	0.8	0.4	0.67	0.47	0.83	0.9	0.67	0.47	0.53
P. notatum	ZI	15	12	20	-	15	14	12	24	14	12	15	10	20	15	15
	AI	0.75	0.6	1	-	0.75	0.7	0.6	1.2	0.7	0.6	0.75	0.5	1	0.75	0.75
T. viride	ZI	22	14	17	-	16	18	14	19	13	15	16	12	20	12	14
	AI	0.62	0.4	0.48	-	0.45	0.51	0.4	0.54	0.37	0.42	0.45	0.34	0.57	0.34	0.4



(a)



(b)



(c)

Fig 1: Comparative graphs of activity index of various extracts on different bacterial strains (a) seed (b) root (c) Leaves

2.5 MIC Evaluation

The chloroform and spirit extract of the plant *C. intybus* were evaluated for determining the minimum concentration effective in showing inhibition of any visible growth of bacteria termed as the minimum inhibitory concentration. For the purpose 5 different concentrations (100µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml) of extract has been prepared and tested for the exhibiting zone of inhibition around them.

Table 2: MIC determination of Chloroform and Methanol samples of roots leaves and stem at concentrations ranging from (6.25 to 100 mg/ml)

Name of the Fungi	Minimum Inhibitory Concentration					
	Root		Stem		Leaves	
	Spirit (mg/ml)	Chloroform (mg/ml)	Spirit (mg/ml)	Chloroform (mg/ml)	Spirit (mg/ml)	Chloroform (mg/ml)
A. niger	98.2±1.63	49.7±2.21	25±5.52	75±6.52	23.5±1.25	12.84±2.92
A. alternata	12.5±1.24	3.63±2.54	6.25±3.6	22.91±2.68	12.5±2.65	6.25±1.28
P. notatum	3.63±1.0	11.65±3.48	47.5±0.92	21.48±3.42	3.63±1.29	10.78±2.88
T. viride	100±1.38	88.73±5.41	41.67±4.7	41.2±3.83	80.2±1.26	60.1+3.44

Values are mean ± SD of three separate experiments.

2.6 Phytochemical analysis

Phytochemical screening of the extracts was carried out according to the methods described below for the detection of active components like saponins, tannins, alkaloids, steroids, flavonoids.

2.6.1 Test for Proteins

Various extracts were dissolved in few ml of water and treated with equal volume of 5% solution of sodium hydroxide and 1% copper sulphate was added. Appearance of pink or purple color indicates the presence of protein in the tested solvent extract.

2.6.2 Test for Carbohydrates

Presence or absence of Carbohydrates in the extract were analysed by Fehling's test. This is a type of reduction test of carbohydrates. Fehling's solution contains blue alkaline cupric hydroxide solution, heated with reducing sugars gets reduced to yellow or red cuprous oxide and is precipitated. Hence, formation of the yellow or brownish-red colored precipitate helps in the detection of reducing sugars in the test solution.

2.6.3 Test for Tannins

To each solvent extract add few drops of ferric chloride solution, pale yellow to brownish black color will appear, determining the presence of tannin in the sample

2.6.4 Test for Phenols

Small quantity of various extracts were taken separately in water and tested for the presence of phenolic compounds with dilute ferric chloride solution (5%). Appearance of violet color in sample shows the presence of phenol in the sample.

2.6.5 Test for Flavonoids

Take 2 ml of different solvent extract in test tubes, add few fragments of magnesium ribbon, then add concentrated hydrochloric acid drop wise, appearance of reddish color shows presence of flavonoids and absence of any color shows that no flavonoid is there in the sample.

2.6.6 Test for Phytosterol

The different solvent extracts were mixed with 2 ml of acetic anhydride. To this mixture 1 or 2 drop of concentrated sulphuric acid was added slowly along the sides of the test tubes. A series of color change shows the presence of phytosterols.

2.6.7 Test for Alkaloids

Take different solvent extracts and acidify them in 2% HCl for 2 hrs, then take 2 ml of each acidified solvent extract and add 2-3 drops of Wagner's reagent. The formation of white precipitate indicates the presence of alkaloids in the sample.

2.6.8 Test for Saponins

Take 2 ml of solvent extract, add some water and shake well, formation of persistance foam indicates the presence of saponins, in the solvent extract.

Table 3: Qualitative test for determining the composition of crude extract of different parts of plant

Plant parts	Extracts														
	Pet ether			Chloroform			Acetone			Spirit			Aqueous		
	R	S	L	R	S	L	R	S	L	R	S	L	R	S	L
Consistency	Br	Y	G	Br	Or	G	Br	Or	G	Br	Y	Br	Br	Y	Br
Proteins	+	+	+	+	+	+	+	++	+	+	++	+	-	-	-
Carbohydrate	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+
Tannins	-	+/-	+	-	-	-	-	-	-	++	+	+	+	+	+
Phenols	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
Flavonoids	-	-	-	-	-	-	-	-	-	+	+	++	+	+	+
Phytosterols	++	++	+	+	+	++	+	+	+	-	-	-	-	-	-
Alkaloids	-	-	-	+	+	+	+	+	+	++	++	+	++	+	+
Saponins	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+

3. Results and Discussion

In the present study, the Antimycotic potential of the various extracts of different plant parts have been investigated and the results have been tabulated in Table 1 and the results of MIC analysis were tabulated in table 2. The results of the phytochemical analysis have been tabulated in table 3.

The phytochemical analysis of extract of root, seed, leaves shows presence of active phytochemical components such as alkaloids, flavonoids, saponins, tannins, steroids. Phytochemical analysis revealed that important chemicals like alkaloids, flavonoids, tannins, saponins, anthraquinones, steroids and terpenoids were present in *Cichorium intybus* seeds. The presence of flavonoids and Tannins has also been analysed in leaf and root extract of *C. intybus.*

The results of agar well diffusion method showed the presence of moderate amount of activity against growth of *Aspergillus niger*, *Trichoderma viride* fungal strains. Whereas, significant amount of antifungal activity has been exhibited by the seed spirit extract, chloroform extract of root and leaf, and the pet ether extract of leaf against *Alternaria alternata*. On the other hand, significant antifungal activity against *Penicillium notatum* was exhibited by pet ether extract of all plant parts (root, seed, and leaf), spirit extract of root and leaf, water extract of seed and leaf.

There are present various reports on the antifungal, antiviral, antimicrobial, antimalarial and nematicidal potential of the plant *C. intybus* [14, 15]. The sesquiterpene lactones present in the plant extracts were suspected to be a contributory factor to its Antimycotic potential. The anomalous extrusion of materials out the wall observed by SEM in *T. tonsurans*, var. *sulfureum*, treated with the *C. intybus* extract, is similar to that previously observed in other dermatophytes treated with chemical synthetic antifungal substance [16] or other plant-derived antimycotics [17]. The plant has good antibacterial and antifungal potential and can be further used for controlling infection and in treatment purposes [18].

4. Conclusion

Plants remain the major source of extracting the potentially valuable chemical constituents for the development of therapeutic agents. The present study of plant provided the information that the root, leaf and seed fraction of plant has significant Antimycotic potential. It clearly suggests that different solvent soluble fractions of plant vary in their fungus growth inhibitory potential. Thus, *C. intybus*is recommended as a significant medicinal plant and can be used in further biological assay.

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