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Phytochemical and Antibacterial screening of *cichorium intybus* seeds use in traditional medicine systems in Pakistan

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Abstract – In human health care system and herbal medicine therapeutic plants show a significant role. Phytochemical are present in different parts of the plants. *Cichorium intybus* is one of the most significant therapeutic herbs belonging to family *Asteraceae.* The present study, elaborates phytochemical & antibacterial analysis of *cichorium intybus* seeds by agar well diffusion method by using Ethanol, Chloroform, Hexane & Aqueous extracts. *S.aureus* and *E.coli* were used as tested bacteria in this study. All the seeds extract of *C.intybus* plant was showed activity against both organisms but Aqueous extract were more active and show large inhibition zone against *S. aureus.* Hexane, ethanol & chloroform extracts show significant zone of inhibition against both organisms. The finding of this study suggests that the medicinal plant has significant antibacterial activity and can utilize for the treatment and control of infection.

Keywords – *Cichorium inttybus*, antibacterial, phytochemical, traditional.

1. Introduction

About half of population of the world for their primary health care depends mostly on conventional drugs [1]. Therapeutic plants were the backbone of conventional medicine system. In last few years pharmacological studies of different plants have been increase [2]. As resistance to most of antibiotics is increasing day by day there is a fundamental requirement to buildup new and innovative antimicrobial medications [3]. This international attentiveness in therapeutic herb reflects identification of the viability of various conventional claims with respect to the use of herbal medicine in treatment of disease & increasing of microbial resistance to the available antibiotics. In past time numbers of natural drug have been used for the cures of bacteriological infection and numerous research works were accomplish in hunt of an appropriate herbal medicine which can efficiently control this infection [4].

In Unani and ayurvedic medicine system the seeds of *cichorium intybus* have been efficaciously used. It is one most important therapeutic plant which belongs to family *asteraceae*. *C.intybus* is a tiny biennial perpetual fragranced plant that carries large number of medicinal comounds i.e. esculin volatile compounds insulin, caumarins, flavonoids and vitamins It carries large amounts of medicinal important phytochemical like volatile oils, saponins, alkaloids, fatty acids, carbohydrate, triterpenoids, tannins and flavonoids etc [5]. The root of this plant contains sesquiterpene like lactucopierin and lactucin, flavonoid like cichoric acid, caffeic acids, quercetin 3 galactose, inulin, phlobaphenes, choline, stable oil & reducing sugar. The seeds contain intybusoloid & triterpenois cichoridiol beside with eleven recognized compound like betulinic acid, syringic acid, vanilic acid, lupeol, beta sitosterol, fridelin, betunaldehyde & sigmasterol, the explosive component includes pentasalicylate, pentadecanone, octane, hexadecane & n-nanodecane. Study publicized that chicory possesses hepatoprottective, anticarcinogenic, hypoglycemic, and anti-ulcer activitys [4].

Escherichia coli is facultative anaerobe rod shaped gram negative bacterium. In warm blooded organisms that are found in lower intestine [6]. It can cause urinary tract infection, neonatal meningitis, gastroenteritis. It is also censurable for septicemia, mastitis, peritonitis, gram negative pneumonia & hemolytic uremic syndrome [7].

Staphylococcus aureus is gram positive cocci. *S.aureus* attains grape-like clusters. It can be differentiated from streptococcus on the basis of catalase and coagulase biochemical tests. In immune compromised patients Staphylococcus aureus is the major origin of skin infections. It also cause meningitis, pneumonia, osteomyelitis and toxic shock syndrome in female, [8]

2. Materials and Methods

2.1. Collection of Plant Materials

The *Cichorium intybus* was collected from the district Dir. and transferred to the laboratory for further processing washed two to three times carefully through flight tap water and one time through germ-free distilled water, and then dehydrated under air shadow. After absolute shadow drying they were cut into small pieces and then grinded in blender. The grind was reserved in small bags of plastic with proper labeling the grand power of plant was used for preparation of different extract [9].

2.2. Preparation of Plant Extracts

Ethanol, chloroform, aqueous and hexane were used as a solvent for preparation of extracts 1000gm dried grind powder of plant was submerged into four liters of all chosen chemical in 8 liter air tense jar and allowed it for

21 days. On daily basis all containers were vigorously agitated for five minutes twice a day that permitted whole process of extraction. The plants extract was than filtered through sterilized filter paper filtrates were kept in air fitted bottles for advance examination [10].

2.4. Phytochemical Analysis

Extract remained verified for the presence of active phytochemical componints like alkaloids, Protein, carbohydrates, bufadienoloids, resins, flavonoids, tannins, Saponins, phenolic compounds, Terpenoids, Steroids, pseudotannins or gallotannins by standard procedures [11].

2.5. Antibiotic susceptibility testing

The susceptibility of frequently used antibiotics to the bacterial isolates was determined by using disc diffusion method on nutrient agar (NA). The consequences were occupied as Sensitive (S) Resistant (R) and intermediate sensitive (I) as describe by CLSI guidelines [12] (CLSI 2007)

2.6. Antibacterial action of herb extract

Two bacteria *S. aureus* and *E. coli* were chosen for antibacterial analysis of *C. intybus* plant extract. antibacterial activity of *C. intybus* plant extract against the selected MDR were determine by agar well diffusion method on nutrient agar media In every Petri plate all four extracts i.e. Ethanol, Hexane, Aqueous and Chloroform of each plant were put into four wells and DMSO were poured in the fifth well as a -ive control. Antibiotic disc (Imipenem) was positioned in the middle of media Petri plate as a positive control. After that every Petri plates were place in an incubator for 24 hours at 37^{o}C. While the completion of 24 hours incubation examine the plats and measuring the zone of inhibition around the wells in millimeter using scale and note the results [13].

2.7. Determination of MIC

The method of MIC as described by kowser and Fatema [14] used to find quantitative value of plant extracts. The process of MIC was carried out in a 15 ml cover test tube having 10 ml nutrient broth media. Plant extract were diluted in nutrient broth media and make different concentration started from 6.25, 12.5, 25, 50 up to 100mg/ml. after that 10 micro liter overnight fresh broth bacterial culture was fixed to MacFarland 0.5 standard were seeded an every test tube. Broth + extract without bacterial culture considered negative control and broth + bacterial culture without plant extract was considered positive control prepared in test tube. All the tubes including negative and positive control were placed at 37 ^{0}C incubated for 24hrs. Then the results were recorded. The minimum concentration able to stop any visible bacterial growth was considered MIC for specific bacteria [14].

3. Results and Discussion

The current study was performed to screen out the phytochemical ingredient and antibacterial activity of *chicorium intybus* seeds. For this purpose chloroform, ethanol, hexane and aqueous extracts were prepared.

3.1. Phytochemical Screening of extract

Table 1 phytochemical composition of Cichorium intybus seeds

No	Phytochemical Componds	Aq	C	E	H
1	Saponins	+	+	+	+
2	Steroids	-	-	-	-
3	Tannins	-	+	+	+
4	Triterpenoides	+	+	+	+
5	Pseudotannins	-	+	-	+
6	Proteins	+	+	+	+
7	Phenolics	-	+	-	+
8	Resins	+	+	+	+
9	Gallotannins	+	-	+	-
10	Flavonoides	+	-	-	-
11	Charbohydrates	+	-	+	-
12	Bufadienoloides	+	+	+	-
13	Alkaloides	+	+	+	+

+ presence, - absent, Aq Aqueous, C Chloroform, E Ethanol, H Hexane

Table 2 Evaluation of antibiotic susceptibility of MDR of selected Bacteria

Antibiotics	Zone of inhibition in millimeter

	Escherichia Coli			*Staphylococcus aureus*		
	S	R	I	S	R	I
Trimethoprim/Sulphamethoxazole	-	8±1	-	0	-	-
Nitrofurantoin	17±1	-	-	-	8±1	-
Ciprofloxacin	21±0.5	-	-	-	8±0	-
Amikacin	17±0.5	-	-	-	10±0	-
Chloramphenical	-	10±0	-	-	8±0	-
Doxycycline	15±0.5	-	-	16±0.3	-	-
Erythromycin	-	-	-	23±0	-	-
Gentamycin	16±0.5	-	-	-	8±1	-
Tetracycline	15±0.5	-	-	-	7±1	-
Tobramycin	-	12±0	-	-	8±1	-
Amoxicillin	-	7±1	-	-	0	-
Amoxycillin/Clavulanic acid	-	7±0.5	-	-	7±1	-
Ampicillin	-	6±1	-	-	6±1	-
Aztreonam	-	7±	-	NI	NI	NI
Cefepime	-	13±0.3	-	16±0	-	-
Cefixime	20±0.5	-	-	-	8±0	-
Cefotaxime	-	16±0.5	-	-	8±0	-
Ceftazidime	-	13±0.5	-	-	-	16±0.3
Ceftriaxone	-	17±0.5	-	-	8±0.5	-
Cefoperazone	-	8±1	-	-	7±1	-
Fosfomycin	25±0.5	-	-	NI	NI	NI
Imipenem	24±0.3	-	-	-	-	15±0.7
Meropenem	-	15±0	-	-	7±0	-
Oxacillin	NI	NI	NI	-	0	-
Pipracillin/Tazobactam	-	-	15±0.3	-	-	14±0.3
Vancomycin	NI	NI	NI	-	7±1	-

+Error of given value, S= sensitive, R=resistant, I= intermediate NI = not indicated

3.2. C. intybus plant extracts Antibacterial against selected MDR

C. intybus chloroform, ethanol, hexane & aqueous extracts were microbiological screen invitro against selected multi drug resistance *E.coli & S. aureus.* as compare to ethanol, chloroform, hexan extract the activity of aqueous extract was high against both bacteria. Against *S.aureus* aqueous extract show 15mm zone of inhibition and against E.coli 14mm inhibition zone were recorded. The zone of inhibition of *C.intybus* chloroform hexane & ethanol extract against *S.aureus* were recorded 12mm, 14mm, and 12mm respectively. Against *E.coli* Hexane, ethanol and chloroform showed inhibition zone in the range between 10mm to 12mm. All inhibition zones were given in the Table 3.

Table 3 Antibacterial activity of Cichorium intybus plant extract against MDR

MDR	Aqueous extract	Ethanol extract	Chloroform Extracts	Hexane extract	+ive IPM	-ive DMSO
E.coli	13±0.3mm	10±0.5mm	10±0.5mm	11±0.5mm	19±0.5mm	0mm
S.aureus	16±0.5mm	11±0.5mm	11±0.5mm	14± 0.5mm	11±0.7mm	0mm

IPM show imipenem positive control, DMSO show dimethyl sulfoxid negative control and + show error in given value

3.3. Minimum inhibitory concentrations of Cichorium intybus.

C.intybus plant aqueous extracts prevent growth the *S.aureus* at lowest concentration of 6.25mg/ml and 12.5mg/ml minimum concentration prevents the growth of *E.coli.* The growth *S.aureus,* and E.*coli* was prevented by chloroform extract at 25mg/ml minimum concentration. Extract of ethanol and hexane prevented the growth of both isolates at 6.25mg/ml and 25mg/ml minimum concentration

Table 4 Minimum inhibitory concentrations of Cichorium intybus.

MDR	Aqueous Extracts mg/ml	Ethanol Extract mg/ml	Chloroform Extract mg/ml	Hexane Extract mg/ml
E.coli	12.5	6.25	25	25
S.aureus	6.25	6.25	25	25

As used both organic solvents and water for extraction of active components of cichorium intybus seeds extracts. The result of this study showed that both organic and water extract of cichorium intybus were active against the selected MDR bacterial isolates that caused different diseases. Bacterial activity of *C. intybus* was may be due to many active components like flavonoids, carbohydrate, alkaloids, bufadienoloides, Pseudotannins etc.

The biological active component was screen by different chemical analysis.

Research papers are present on the anti-inflammatory, anthelmintic, antimolluscal, antiviral, antifungal, and antibacterial activity of plants. Some of these researches were useful in identification of active components for such activity and for developments new drug for the treatment of human infection [15]. The antibacterial activity of *C. intybus* ethyl acetate, ethanol and water extract was testified against *P. fluorescens*, *P.aeruginosa*, *E.carotovora*, *A.radiobacter*. [16]. In 2013 verma *et al* [17] isolated Lactucopicrin & Lactucin from *C.intybus* and described for its antimalarial & antibacterial action. Study exhibited that the leaves & roots of *C. intybus* retain durable nematicidal & antibacterial activity. However very limited studies is existing on antimicrobial properties of *C. intybus* leaf & roots against the most significant human pathogens.

4. Conclusion

The currents study elaborated the antibacterial action of different extract of *C. intybus* against *E. coli & S. aureus*. The result of current investigation indicates that the antimicrobial action diverge with extract of plants. The results of current recommend that the plant has respectable antibacterial activity and can be utilize for the treatment and control of disease.

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