Investigation of Antimicrobial and Antioxidant Activities of *Cirsium arvensis* (L.) Scop From District Bhimber of Azad Jammu and Kashmir

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Summary: Plants are part and parcel of human life. The current research was designed to explore ethnomedicinal importance of "*Cirsium arvensis* (L.) Scop." (family Asteraceae) from Samahni area of District Bhimber Azad Kashmir, Pakistan. The maceration protocol was applied using four solvents of different polarity and highest yield was found in water extract for leaf (9.85 mg) and for stem (8.50 mg) from 250 g dry wt of sample. Leaf extract produced significant quantity of biochemicals: saponin, terpnoids, alkaloids and tannins. The antimicrobial activity was explored using four bacterial strains and four fungal species. Petroleum extract (P.E.) macerate depicted the highest antibacterial and antimycotic activity. P.E. extract of leaf showed significant zone of inhibition (Z.I.) nearly 76±0.4 mm which was the most promising. For antimycotic, highest ZI was calculated for cholorformic extract of leaf (79±0.2 mm) against *Aspergillus niger*, followed by 77±0.7 mm for *Fusarium oxysporum*. Minimum inhibitory concentration (M.I.C.) was also determined for different bacterial and fungal strains. It was found 0.12±0.05 M.I.C. for *E. coli* and followed by 0.26±0.3 mm for *B. subtilis.* For antimycotic analysis, M.I.C. was determined significant for *Aspergillus niger* (0.05±0.3). In the study, activity index (A.I.) for bacteria was highest (4.22) for P.E. of leaf extract against *E. coli* and for fungi it was highest (3.59) for leaf chloroform extract against *A. niger*. The plant crude extract was also tested for total antioxidant activity (TAA) and it depicted 4.70±10.8 value for aqueous macerate. Other fractions also do have good range of TAA. In total phenolic content (TPC) analysis, highest TPC was determined for methanol extract of stem of for concentration of 125 mL with value of 78.93±0.3. These findings proved that plant is good source of herbal medication and it might be good source of novel drug discovery and development against bacteria and fungi.

Keywords: Samahni, Antimicrobial, Antioxidant, *Cirsium arvensis*, Azad Kashmir, Activity Index, MIC

Introduction

Plants are inevitable part of human life since his emergence on this planet. These plants are being used by human for different needs of his life such as food, fodder, medicines, fuel, and shelter. Around the globe, plants are being used as source of food and medicines as two main use values [1]. More than 7000 plants have been used for source of medicines in different traditional medical systems. In many developing countries of world more than 80% population is relying on the wild herbal medicines [2]. The botanic drugs have been ubiquitously used around the globe because these herbal medicines are cost effective, good efficacy, and comparatively with nonth or less side effects. Recently 88% of world population is inclined towards use of herbal medicines for maintaining health and combating different diseases. World health organization (WHO) recognizes that plants are the best source of extraction of variety of drugs [3].

Human beings are ubiquitously infected due to universal prevalence of different microbes in the environment. Due to unhygienic conditions and malnutrition, the rural populations of world are being infected by various strains of bacteria and fungi causing different diseases. As Antimicrobial activities of plants have been potential source of combating fungal and bacterial pathogens from pre-historic times. Hence, recently, plants are being screened fastly in different research institutes and pharmaceutical industries for extracting novel single or group of compounds for controlling or eradicating of microbial and infectious ailments [4]. Many pathogenic diseases are being controlled by retarding their growth or killing using different allopathic drugs but there is tendency of resistance development in different bacteria and fungi, so there is dare need of discovery of potential, active and effective compounds for fighting their resistance property. Many bacteria have ability to transmit their resistant genetic property to next generations and and acquire further resistance to synthetic drugs which are being utilized as therapeutic agents [5].

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Many pharmaceutics and researchers have conducted comprehensive investigations on the medicinal plants for the investigation and production of different effective drugs. Chemotherapy is under continuous search and formulation of new drugs which will counter the challenges posed by resistant strains of microorganisms. The investigation of indigenous plants for the purpose to evaluate the antioxidant and antimicrobial properties and these plants may yield useful results. Different parts of world are rich hub of plant biodiversity and many of those are used in traditional medicines such as in Africa, Europe and Asia too [6].

Plants rich sink of natural phytochemicals possessing property of antioxidants are being used in lessening and reducing aging effects and other old-age diseases and also these useful in promotion of general and potency health paradigms. Among natural antioxidants commonly used are flavonoids and phenols which being are being obtained from natural plants in form of various botanic drugs [7]. Antioxidants are very effective against many degenerative diseases such as heart disease, paralysis, cancer, diabetics, alzheimer and parkinson diseases, etc. The natural antioxidants such as flavonoids, isoflavonoids, flavonons, tocopherols, saponins, carotenoids, vitamin C and other phenolic compounds are present in many plants described as medicinal plants [8].

In the current study, wild plant *Cirsium arvense* (L.) Scop. of family Asteraceae was selected for analyzing antimicrobial and antioxidant activities. The family has more than 1620 genera and 23600 species of herbs, shrubs, and trees distributed throughout the globe and some of its species such as *Cynara scolymus* has high content of total phenols i.e. 45.11 and 37.00 mg of flavonoids [9]. This plant has been used for source of ethnomedicines in local inhabitants of the area.

Pakistan is diverse country with dynamic climate and earth features. It bears huge diversity of more than 6000 plants species and out of these many are used in daily life as source of food, fodder and/or medicines in life of people [2]. Up to now, 600-1000 plants are studied pharmaceutically and declared as medicinal plants hitherto many taxa are awaiting for disclosure of their status [10]. Many of these explored species are used as source of medicine and traded in local and international markets. The local rural people of the area collect the plants from native forest areas for their domestic uses and some of it sell out to local stakeholders or direct send it to main cities through brokers to earn money [11].

Samahni is the one of the lush green and beautiful of District Bhimber, Azad Jammu and Kashmir. The area is rich hub of many medicinal plant species and many of these are yet to be explored for ethnopharmacological potential. Particularly plants present near the buffer zone of line of control (LOC); a border area between Azad Kashmir and Indian Occupied Kashmir are thickly populated because this area is the least disturbed area by anthropogenic interferences. The study area is rich source of pine forest and many species are used for different purposes by local inhabitants [12]. In the area many indigenous plants are used by local healers for curing many diseases in hilly and remote mountainous areas because of less access to urbanization and easy availability of wild medicinal plants and their by-products.

Major objectives of the research were: (1) to study antibacterial activity of *C. arvensis* (L.) Scop. in different solvents and explore its minimum bactericidal concentration (MBC), (ii) to study antifungal activity of *C. arvensis* (L.) Scop. and find its minimum fungicidal concentration (MFC) (iii) to study antioxidant activity of *C. arvensis* (L.) Scop. and to (iv) to determine zone of inhibition (Z.I.) and activity index (A.I.) for sampled parts in four types of solvents for comparison.

Experimental

Plant Material

The plant samples of *Cirsium arvensis* (L.) Scop. were collected from different areas of Tehsil Samahni Azad Kashmir. The plants were authenticated, assigned accession number (MUST-5343) and their herbaria were deposited in Herbarium of Department of Botany (MUST) Bhimber Campus for future reference. The experimental plant samples were shadow dried for further research work.

Test Organisms

For bacterial test organisms used for evaluation of antimicrobial activities of *Cirsium arvensis* were collected from Department of Biotechnology and Microbiology Lab of Medical College Mirpur. Following strains of bacteria were used for analysis.

Bacillus subtilis (ATCC6633)*:* It is a gram-negative bacterium which causes a diseases named as dermal allergy in individuals. It also causes urinary tract infection (UTI).

Pseudomonas aeruginosa (ATCC27853): It is a gram-negative bacterium which cases diseases such as softening of tissues, gastrointestinal disease, septic shock and several skin infections.

Staphylococcus aureus (ATCC25923): It belongs to group of gram-positive bacterium and it is causative agent of many ailments and infectious diseases such as meningitis, pneumonia, toxic shock syndrome (TSS) and endocarditis.

Escherichia coli (ATCC25922): It is gram-positive bacterium which causes bacteremia, urinary infections and cholecystitis disease. Its prevalence in human body cause paleness and anemia; and sometimes its occurrence in kidney might collapse its functionality and kidney failure happens.

Aspergillus niger: Its prevalence is cosmopolitan. It can grow on many substrates because needs few micronutrients for its life sustenance. If it infects human body then it may develop asthma and allergy. Its infusion in ears may damage its internal soft parts and leading to permanent deafness. *Aspergillosis* is also common in human being that is also due to infiltration of this fungal taxon.

Aspergillus oryzae: It is toxic species and mycotoxin produced causes damage pulmonary arties and veins causing diseases called *aspergilloma*.

Fusarium solani: It infects and attacks on nail and also causes onychomycosis disorder. It also causes sinusitis ailment and keratitis is also produced by it. It causes diseases in cereal corps' grains.

F. oxysporum: It infects and attacks on nail and also causes onychomycosis disorder.

Plant Sample Collection

Leaf and stem parts of healthy plants were Tehsil Samahni area. Mature leaves and fresh emerging stem pieces were collected in polythene bags for experiment. Fresh complete plant specimens were also collected for herbaria preparation, which were deposited in Department of Botany, in University for future reference. The experimental plant parts were brought to lab and shadow dried.

Extraction of Plant Materials:

The research investigations were conducted in light of established protocol which comprises of following steps: dried plant parts cut into pieces and then ground to powder form by commercial grinder into fine powder of 1mm size. Ground plant material was macerated in four non-polar to polar solvents. The plant materials of 250 g of each sample were soaked in 250 ml of Petroleum ether (PE) in beakers or jars and tightly sealed. The dipped plant material was left it over for one week to be dissolved at room temperature (RT) and then filtered using whatman filter paper no 42 [13]. The residue was again submerged in next solvent s viz: Chloroform, methanol and water for seven days at RT, each. The process was repeated for all solvents. The filtrate was evaporator in rotary evaporator and then dried material was measured in mg for its extract yield estimation. Then % age yield of the each plant was estimated and presented in tabular format. The %age yield was calculated by using this formula [13]:

$$\% \text{ of extract yield (ug)} = \frac{\text{Weight of plant extract (g)}}{\text{Weight of initial plant sample (g)}} \times 100$$

Prior to determination of antimicrobial and antioxidant activities general physical properties of plant extract were noted which included form, percentage yield texture and color.

Phytochemical Tests

Comprehensive quantitative and qualitative analyses were performed and their results were tabulated in proper format. In qualitative tests, the presence and absence of different chemicals: saponins, flaovnoids, flavonons, terpenoids, and alkaloids were found using various lab tests as per protocol of Ayoola *et al*.,[14] while in quantitative tests concentration of different compounds was determined using protocol of Harbone [15] with bit modifications.

Antimicrobial Activity

The antimicrobial activity of different fractions of plant were determined by using protocol of agar well diffusion method according to Ferreira *et al.*, 2001 [16-21]. The minimum bactericidal concentration (MBC) was estimated by making serial dilutions and its sub-cultivation in microliter plates (MLPs) with mixture of 2 µl of sample and 100 µl of nutrient broth (NB) per well and the MLPs were incubated in incubator keeping constant temperature of 25 ^{o}C for 3 days. The least concentration with depicting none of visible growth of bacterial colonies was declared as MBC, showing 99.9% of killing or retardation of original inoculum used. Optical density (OD) of each well on media was determined by using digital colony counter (Germany) and its results were compared with standard readings of Ampicillin used as positive control in the trial. In these experiments, blank solvents were employed as negative control in all sample analyses and data were tabulated in and

these controls were used to know any antimicrobial power of only pure solvent (blank). All experiments were repeated in thrice and subjected to statistical analysis for authenticity [22].

Antimycotic Activity

The minimum fungicidal concentration (MFC) was determined by agar well method, as used above. MFC was calculated by making serial dilutions and its sub-cultivation in microliter plates (MLPs) with mixture of 2 µl of sample and 100 µl of potato dextrose agar (PDA) medium per well and the MLPs were incubated in incubator keeping constant temperature of 29 ^{o}C for three days. The least concentration with depicting none of visible growth of bacterial colonies was declared as MBC, showing 99.9% of killing or retardation of original inoculum used. Optical density (OD) of each well on media was determined by using digital colony counter (Germany) and its results were compared with standard readings of Penicillin was used as positive control in the trial [23]. All experiments were repeated in thrice and subjected to statistical analysis for authenticity.

Calculation of Activity Index (AI)

Activity index (AI) of samples was calculated by using values of MIC which was determined prior to find out antimicrobial potential of all the plant parts with four types of solvents [24]. The AI was calculated using following formula:

$$\text{Activity Index (AI)} = \frac{\text{Zone Inhibition (ZI) values of Sample}}{\text{Zone Inhibition (ZI) values of Standard}}$$

Determination of Antioxidant Activity

The antioxidant activity of different extracts prepared from two plant parts of *Cirsium arvensis* were determined by protocol of Lee and Shibamoto with bit modifications [25]. DPPH was used radical scavenging assay for different extracts and compared with results of standard antioxidant butylated hydroxytoluene (BHT).

Stock Solution Preparation

For this analysis, 0.02 g of dried/solidified extract of each of solvent was dissolved in 10 mL of methanol in a cylinder and its final volume was made 20 mL by adding more methanol quantity.

Preparation of Dilutions Series

The each of extract was dissolved in above-mentioned method and then its further dilutions were prepared using series of 125 µl, 250 µl, 500 µl, and 1000 µl. The absolute volume of DPPH assay conducted was made 500 µl and then its reading was taken.

Preparation of 0.1 mM DPPH solution

A cylinder of 1 L volume was taken and ca. 0.04 g of DPPH was taken and dissolved in it using 100 mL of methanol as solvent. Then its final volume was maintained up to 1 L by adding more methanol. For analysis following 60 µl, 125 µl, 250 µl and 500 µl dilutions were prepared as shown in Table-1. The DPPH having concentration of 0.1 mM can be processed and stored at 4°C for further use. But it important to prepare fresh solution of DPPH and use it for better readings and results. sample was got by macerating it in 1000 µl of water with consistency as: 250 µl stock solution of plant + 750 µl methanol and it was mixed and its final volume was made up to 3 mL by using DPPH solution. This solution was agitated and then kept it for stand for 40-60 min in RT. The same procedure was employed for all the tubes and tests. During this time zone of incubation, reduction reaction of DPPH had taken place and its color of indication was changed from purple to yellow that is its end point.

Total antioxidant activity (TPA) fractions of of plant extracts was determined by application of methodology as described in previous literature [26]. In the analysis Total phenolic content (TPC) of was calculated using the method described by Makkar *et. al.,* with some modifications [27].

Results and Discussion

In the study, maceration technique was applied for extraction of bioconstituents from dried samples of *Cirsium arvensis* (L) Scop. Four different solvents with variable polarity were used for extraction. Leaves sample produced high yield of 9.85 mg/g in aqueous medium while least extraction yield (1.95 % was obtained in stem macerate using petroleum ether solvent (Table-1). The chemical profile of stem and leaf extracts of *Cirsium arvensis* was formulated by maceration process using four solvents i.e. petroleum ether, chloroform, methanol, and double distilled (d.d.) water. Different phytochemical tests were used for qualitative and quantitative analysis of stem and leaf parts of the plant. It was found that leaf has comparatively more quantity of analyzed compounds as compared with stem part (Table 1). These findings are coinciding with previous research findings of the researchers, because leaf is the most active part of plant where photosynthesis occurs [28].

Table-1: Qualitative Phytochemical Analysis of Leaf and Stem Macerates of *Cirsium arvensis* (L.) Scop.

Constituents	Phytochemical tests	Presence of different Chemicals in extracts			
		Petroleum ether	Chloroform	Methanol	Aqueous
Terpnoids	Salkowski test	+	–	+	+
Flavonoids	$FeCl_3$ test	–	–	+++	++++
Saponins	Frothing test	++	+++	++++	++
Alkaloids	Mayer's test	++	–	+++	–
Tannins	$FeCl_3$ test	++	+	+++	++
Cardiac glycosides	Keller-Killiani test	+	++	–	+++

Table-2: Profile of Zone of Inhibition (ZI) of Bacterial Strains Produced Against Standard Antibiotic Discs (Positive Control)

Bacterial Strains	Concentration (ug/mL)	Standard Disc	Zone of Inhibition (mm)	Final Response
E. coli	20	Amikacin	18±0.5	Resistant
P. aeruginosa	20	Cephalaxin	13±0.7	Resistant
S. aureus	20	Erythromycin	24±1.5	Resistant
B. subtilis	20	Ampicillin	23±0.6	Resistant

Table-3: Zone of Inhibition (ZI) Produced by Standard Antifungal Disc Using Different Antibiotics (Positive Control)

Fungal strains	Concentration (ug/mL)	Antifungal standard disc	Zone of inhibition (mm)	Final Response
A. niger	50	Terbinafine	22±2.0	Intermediate
	50	Griseofulvin	20±1.5	Intermediate
A. flavis	50	Terbinafine	23±2.5	Intermediate
	50	Griseofulvin	21±1.0	Intermediate
C. albicans	50	Terbinafine	22±3.5	Intermediate
	50	Griseofulvin	19±4.5	Intermediate
F. solani	50	Terbinafine	18±2.5	Intermediate
	50	Griseofulvin	19±0.8	Intermediate

Table-4: Zone of Inhibition (ZI) and Activity Index (AI) Produced by Stem and Leaf Extracts of *Cirsium arvensis* Against Four Bacterial Strains

Plant Parts	Extracts	Zone of Inhibition (ZI) in mm and Activity Index (AI)							
		E. coli		*S. aureus*		*P. aeruginosa*		*B. subtilis*	
		ZI	AI	ZI	AI	ZI	AI	ZI	AI
Stem	Petroleum ether	35±0.4	1.94	18±0.3	1.38	17±0.3	0.70	11±0.3	0.47
	Chloroform	27±0.2	1.5	37±0.2	2.84	18±0.4	0.75	17±0.4	0.73
	Methanol	55±0.5	3.05	37±0.5	2.84	36±0.5	1.50	46±0.2	2.00
	Aqueous	11±0.3	0.61	20±0.3	1.53	14±0.5	0.58	10±0.5	0.43
Leaves	Petroleum ether	76±0.4	4.22	55±0.5	4.23	21±0.1	0.87	31±0.3	1.34
	Chloroform	53±0.5	2.94	22±0.4	1.69	72±0.5	3.00	36±0.2	1.55
	Methanol	55±0.3	3.05	42±0.3	3.23	55±0.3	2.29	35±0.1	1.52
	Aqueous	33±0.4	1.83	12±0.5	0.92	15±0.4	0.62	20±0.5	0.86
Standard (Ampicillin		18±0.5		13±0.7		24±1.5		23±0.6	

Table-5: Zone of Inhibition (ZI) and Activity Index (AI) Produced by Stem and Leaf Extracts of *Cirsium arvensis* Against Four Fungal Strains.

Plant	Extracts	Zone of Inhibition (ZI) in mm and Activity Index (AI)							
		A. niger		*A. oryzae*		*F. oxysporum*		*F. solani*	
		ZI	AI	ZI	AI	ZI	AI	ZI	AI
Stem	Petroleum ether	34±0.3	1.54	19±0.5	0.90	18±0.7	0.94	16±0.7	0.84
	Chloroform	26±0.1	1.18	41±0.4	1.95	19±0.3	1.00	18±0.5	0.94
	Methanol	28±0.4	1.27	36±0.3	1.71	35±0.2	1.84	49±0.5	2.57
	Aqueous	13±0.5	0.59	21±0.2	1.00	17±0.4	0.89	13±0.3	0.68
Leaves	Petroleum ether	59±0.4	2.68	24±0.4	1.14	20±0.2	1.05	35±0.6	1.84
	Chloroform	79±0.2	3.59	53±0.4	2.52	77±0.7	4.05	38±0.5	2.00
	Methanol	52±0.2	2.36	44±0.4	2.09	58±0.5	3.05	33±0.3	1.73
	Aqueous	30±0.7	1.36	15±0.6	0.71	18±0.6	0.94	21±0.4	1.10
Standard (Griseofulvin)		22±2.0		21±1.0		19±4.5		19±0.8	

Exploration of phytochemicals very significant because it due to chemical compounds that each plant has defensive mechanism and then products or crude form is used as medicine to combat various ailments. Six different tests were conducted in lab to make profile of presence or absence of the chemicals in the plant material and then amount of these phytochemicals was determined and their values are shown in tabular form. The analysis showed that different compounds viz: terpenoids, flavonoids, saponins, alkaloids, tannins and cardiac glycosides were present in the tested samples (Table-

1). The quantitative findings showed that highest amount was found for flavonoids with 17.50±1.2 values in leaf macerate and least extraction was obtained for terpenoids with 02.08±0.5 in stem samples (Table-2). The results depicted that in leaf sample; the highest amount (17.50±1.2 mg) of flavonoids and least amount (05.09±0.1) of terpenoids were present. In stem powder extraction process, flavonoids were high in concentration with 14.30±0.3 values and terpenoids yielded 02.08±0.5 concentration (Table-2). The high concentration of flavonoids was seen in both samples and similar activity was mentioned in past work of Keser in Turkey reported that antimicrobial and antioxidant activity of kernels of plant (*Prunus dulcis* Mill.) was due to presence of such type of compounds. [29].

There are various methods available for exploration of antimicrobial potential of plant parts but agar well diffusion (AWD) protocol is applied as per recipe of Kirby, 1995 with some modifications. This protocol is good to measure the activity of extract against different cultures of microbial strains [30]. This method is better than disc diffusion method (DDM) because prior one is accurate and very low quantity of sample is required for analysis than later one which requires high amount i.e. 50, 100, 150 ml of samples [31]. The restricted growth of microbes was compared for plant extracts with that of standard antibiotics. For all four solvents (petroleum ether, chloroform, methanol and water) negative controls readings were determined to know their potential as antimicrobial products and findings proved these solvents don't have any antimicrobial activity. The positive controls of plant extracts against standard (antifungal and antibacterial) discs using different antibiotics were employed and comparative analysis was conducted measuring their capacity of zone of inhibition (ZI) as shown in Table (3 and 4), which are in relevance with past work of researchers [32].

In the analysis, antimicrobial potential of leaf and stem parts of the plant were tested by determining antibacterial and antimycotic activity. In the research, it was determined that plant has antibacterial potential. In the antibacterial analysis, four strains of bacteria viz*: E. coli, S aureus, P. aeruginosa* and *B. subtilis* were used. In the study, zone of inhibition (ZI) and activity index (AI) were determined for leaf and stem samples extracted in four solvents against four bacterial strains. It was found stem extract of plant in methanol showed highest ZI (55±0.5) and AI (3.05) against *E. coli* and least values of aqueous extract having ZI with 10±0.5 and AI with 0.43 were explored against *B. subtilis* (Table-5). The antibacterial activity of different extracts were ranging were between 20±0.3 to 37±0.2 against *S. aereus* and 14±0.5 to 36±0.5 against *P. aeruginosa* which corroborates with previous research findings of Hufford *et al.,* [31,32]. In plant leaf sample analysis, P.E. extract depicted maximum values of ZI (76±0.4) against *E. coli* and highest AI (4.23) was found for P.E. extract against *S. aureus* strain. The aqueous extract of leaf showed least AI (12±0.5) values against *S. aureus* and least AI (0.62) was found against *P. aeruginosa* bacterium (Table 5). These findings prove that extracts of relative polar solvents are comparatively better than polar ones and there are same type of past research proves which corroborate our study [29]. From this analysis, the order of resistance of bacterial stains can be generated viz: *E. coli*<*S. aureus*< *P. aeruginosa*< *B. subtilis*. The antibacterial activity exhibited by both parts of plant extracts was greater than standard discs as shown in Table-5. These antibacterial activity might be presence of different types of phytochemicals in extract analyzed and similar findings have be reported by past researchers, that plants do possess good potential for controlling /eradication of bacteria [33]. The past researchers proved that chemical compounds extracted from *Petalostemum purpureum* and *Pulicaria undulata* had shown good potential against *E. coli* and then *P. aeruginosa*. Among the tested solvents, petroleum ether showed maximum (76±0.4 mm) antibacterial activity and aqueous medium showed the least activity values (12±0.5 mm). These results depicted that trend from polar to non-polar Similar types of findings have been reported in past work of Ajaib *et al*., 2014 [33].

The leaf powder of plant *Cirsium arvensis* (L) Scop. depicted better antibacterial potential than stem. It was proved that extract has highest ZI i.e. 76±0.4 against *E. coli*. While, its other fractions obtained from other solvents have shown also better results of ZI than stem part when compared in general (Table-3). However, aqueous extract also showed better value of ZI i.e. 33±0.4, than stem part extract against same test organism. These extracts do possess more potential of antibacterial activity than stem, it is due to the fact the leaf is active part of plant where photosynthesis occurs concurrently and it is called factory of phytochemicals. The presence of different chemicals and their high concentration is proof of its better activity than other plant parts. Similar research reports have been shown in papers by past scientists [34].

In antimycotic analysis, four strains of fungi including *: A. niger, A. oryzae, F. oxysporum* and *F.*

solani which are human pathogens and cause different fatal diseases are selected and tested. In this study, zone of inhibition (ZI) and activity index (AI) were calculated for both parts (leaf and stem) of plant extracting in four solvents using maceration method. It was found chloroform extract of stem part of plant showed highest ZI (41±0.4) and AI (1.95) against *A. oryzae* and least values of aqueous extract having ZI with 13±0.3 and AI with 0.68 were explored against *F. solani* species was determined (Table-5). While in second part viz. leaf extraction it was found that chloroform extract was best one with ZI of 79±0.2 and 3.59 against *A. niger* species. In this leaf extract analysis, aqueous extract was found to be the least effective with ZI 15±0.6 and AI 0.71 against *A. oryzae* (Table-5). The extracts of plant depicted highest antimycotic activity against *A. niger* (79±0.2 mm) and *F. oxysporum* (77±0.7 mm) and this result are in coincidence with past work of Ajaib *et al*., 2015 [34]. The antimycotic profile of stem and leaf parts of plant depict that from this analysis, the order of resistance of bacterial strains can be generated viz: *E. coli*<*S. aureus*< *P. aeruginosa*< *B. subtilis*. The antibacterial activity exhibited by both parts of plant extracts was mostly greater than standard discs except few extracts (Table-5).

In other analysis, minimum inhibitory concentration (MIC) was also determined for petroleum ether solvent against antibacterial and antimycotic strains. The range of MIC was variable and it was 0.04±0.02 mg/mL against *P. aeruginosa* to 0.27±0.04 mg/mL against *E. coli*. It was concluded that MIC of 0.27±0.04 was found for *E. coli* at concentration of 0.9 mg/mL and least response was recorded for *S. aureus* in terms of MIC (0.08±0.5) at concentration of 0.4 mg/mL. The similar significant results were obtained for both media of experiment as described by previous researchers [35]. It proves that plant under investigation do possess good potential for antimicrobial activity.

The antioxidant activity of plant parts was analyzed using four solvents and 1,1-diphenyl-2-picrylydrazyl (DPPH) method was applied for this test. All experiment was repeated thrice for each plant part and each solvent. The data were tabulated and analyzed by using software of minitab and SEM were calculated of the replica (Table-6). The methanolic extract depicted maximum DPPH scavenging activity with values of 78.93±0.3 mg/mL at concentration 125 ml in stem sample of the plant (Table-6). For leaf sample analysis of the plant, highest value of DPPH was found for petroleum ether having 81.72±0.3 mg/ml at conc. of 500 ml while least values were obtained 16.62±0.6 mg/ml at concentration 125 ml (Table-6). These results are similar with past work of researchers as this activity is was higher for methanol extract [36]. These findings were in-line with previous reported work of Bokhari *et al.*, 2013 [37]. The antioxidant percentage of DPPH of leaf and stem of *Cirsium arvensis* (L.) Scop. were accordance with work of reported by Ajaib *et al*., 2013b and Malik *et al.*, 2012 [31-38].

Table-6: % DPPH free radical scavenging activity of stem and leaf of *Cirsium arvensis* (L) Scop.

Plant parts	Solvent/Extracts	Volume Used (µL)	% scavenging of DPPH
Stem	Petroleum ether	500	68.31±1.2
		250	61.97±1.7
		125	45.59±0.3
		50	72.51±0.3
	Chloroform	500	57.11±1.5
		250	81.23±0.5
		125	53.16±0.4
		50	66.24±0.2
	Methanol	500	72.34±1.9
		250	71.93±0.5
		125	78.93±0.3
		50	45.59±0.2
	Water	500	62.88±0.8
		250	61.23±1.6
		125	73.66±1.1
		50	72.73±1.0
Leaf	Petroleum ether	500	81.72±0.3
		250	71.60±0.6
		125	60.24±1.0
		60	57.28±1.2
	Chloroform	500	49.62±1.1
		250	58.18±0.5
		125	16.62±0.6
		60	64.64±0.2
	Methanol	500	42.13±0.3
		250	64.77±1.0
		125	40.65±0.4
		60	55.58±0.4
	Aqueous	500	71.60±1.5
		250	33.16±1.0
		125	81.15±1.0
		60	75.72±1.2

Total Antioxidant Activity (TAA) of stem extracts of *Cirsium arvensis* was determined and it ranged 0.85±0.04 to 4.70±10.8. While TAA of leaf extract was in range of 1.12 ±0.4 to 3.12±0.2 which was higher than standard BHT with 1.118±0.05 (Table-7). TAA was highest in aqueous sample having 4.70±10.8 values while least values were seen in chloroform extract with 0.85±0.04 (Table-8). These findings were similar with previous work on Ajaib et la., 2013c in which he described TAA analysis in *Iris aitchisonii* (Bakar)) Boiss [40]. These findings do prove that the plant is very promising for suppressing oxidants and ROS. It can be used for herbal drug and can reduce or lessen the risk and hazardous of different parameters. These outputs are in coincidence with past research work.

The total phenolic content (TPC) analysis of the stem sample of *Cirsium arvensis* was determined,

it was highest (1.75±0.6) in chloroform solvent extract, and its least values (1.05±0.9) were seen in water extract of plant (Table-8). In other leaf part of plant sample, TPC was maximum in concentration in aqueous extract with 1.17±2.1 mg/ml while it was minimum in analyze of methanol with 0.72±1.5 mg/ml. These former mentioned TPC data were in coincidence with past work of Ajaib et al., [41]. While in later one, it is discussed that aqueous sample produced more quantity of TPC than other solvents and it may be due to more concentration of compounds in leaves that are active part of photosynthesis in the plants. These findings are similar with work of Didier *et al*.,2011 [42] who conducted research on 18 medicinal or food plants of compositae with a claim that leaves have more concentration of phenols and flaovnoids [43]. These compounds are known for curing CVD and other oxidative stresses in past references/works.

Table-7: Total Antioxidant Activity (TAA) of Stem and Leaf extracts of *Cirsium arvensis.*

Plant Material	Extracts	Absorbance at 695 nm
Stem	Petroleum ether	2.40±0.02
	Chloroform	0.85±0.04
	Methanol	1.90±0.05
	Aqueous	4.70±10.8
Leaves	Petroleum ether	3.12±0.2
	Chloroform	1.12 ±0.4
	Methanol	1.60±0.03
	Aqueous	2.25±0.05
BHT	Standard	1.118±0.05

Table-8: Total Phenolic Content of stem and Leaves of *Cirsium arvensis.*

Plant Material	Extracts	Absorbance at 725 nm
Stem	Petroleum ether	1.25±2.5
	Chloroform	1.75±0.6
	Methanol	1.12±2.1
	Water	1.05±0.9
Leaves	Petroleum ether	0.85±0.6
	Chloroform	0.92±0.2
	Methanol	0.72±1.5
	Water	1.17±2.1

These results are indicating that plant is significantly important for its antimicrobial potential and it can be good source of drug development against bacteria and mycotic infections and their diseases. This study proves that the selected plant is good source of herbal medicine and it can be used at industrial scale to produce effective medications with standard and authenticated means.

Conclusions

The phytochemical analysis of leaf and stem parts of *Cirsium arvensis* plant depicted that it contains variable quantity of chemicals like saponins, flaovnoids, terpenoids, alkaloids, cardiac glycosides and tannins. Leaf sample showed more quantity of chemical constituents than stem macerate. The antimicrobial analysis indicated that maximum antibacterial activity was shown by methanolic extract of stem against *E.coli* and in case of leaf sample its antibacterial potential was better in P.E. macerate against *E.coli.* The plant do possess antimycotic activity and it was maximum against *A. niger* in chloroform macerate that is greater than standard antifungal disc. The antioxidant activity was determined by DPPH and it was very significant. Its DPPH scavenging activity was highest for aqueous extract. These results indicated significant antimicrobial activity and antioxidant potential thus supporting that this plant is medicinally very important.

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