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Phytochemical analysis, antimicrobial, antioxidant and enzyme inhibitory activities of ethanolic extract of *Centaurea solstitialis* L. and its different fractions

Saeed Ahmad[1], Sahar Saba[1] & Hafiz Muhammad Asif[2]*

[1]Department of Pharmacy, Faculty of Pharmacy & Alternative Medicine, The Islamia University of Bahawalpur, Pakistan
[2]University College of Conventional Medicine, Faculty of Pharmacy & Alternative Medicine,
The Islamia University of Bahawalpur, Pakistan

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Resistance to conventional antimicrobial regimes is one of the issues of concern in healthcare and it drives the need for development of new antimicrobial agents. Medicinal plants, as rich source of biochemical and bioactive compounds, serve as potential source for new drugs. Here, we evaluated the ethanolic extract of *Centaurea solstitialis* L. and its different fractions (n-hexane, choloroform and n-butanol soluble fraction) for antimicrobial, antioxidant and enzymes inhibitory activities. Antibacterial activity against two Gram-positive and three Gram-negative bacteria species were determined by using agar well diffusion and 96-wells microplate methods. Similarly, antifungal activity against two fungal strains was also evaluated by agar well diffusion method. Antioxidant activity analyzed by measuring the scavenging activity of DPPH radicals and acetylcholinesterase, butrylcholinesterase and chymotrypsin inhibitory activity was determined at 10 µg/mL and 1.0 mg/mL concentrations. Results revealed that the ethanolic extract of *C. solstitialis* and its different fractions possesses significant (P<0.05) antibacterial activity and effective against fungi *Aspergillus niger* and *Macrophomina phaseolina*. Significant (P<0.05) DPPH scavenging activity (88.52±0.23%) among all fractions was noted. n-Butanol fraction showed significant acetyl-cholinesterase (78.55±0.76%) and butrylcholinesterase inhibitory activity (78.1±0.41%) with IC_{50} values of 54.6±0.39 µg/mL and 211.9±0.15 µg/mL, respectively. Maximum chymotrypsin inhibition activity was shown by crude ethanolic extract (87.76±1.17) with IC_{50} value of 38.23±0.75 µg/mL. It is concluded that *C. solstitialis* extract and its fractions possess significant antimicrobial, antioxidant and enzyme inhibitory activity.

Keywords: Acetylcholinesterase, Antibacterial, Antifungal, Antimicrobial, Butrylcholinesterase, Barnabys thistle, Chymotrypsin, DPPH, Golden star thistle, Yellow star thistle

Plants serve as the potential source of food and drugs apart from clothing and shelter since ancient times[1]. Approximately, 70-90% of the world population use medicinal plants as source of medicines against many diseases, and new antibacterial, anticancer, antioxidant[2], hepatoprotective[3], neuroprotective[4], antihypertensive[5], analgesics[6], nephroprotective[7] drugs have been extensively explored from these medicinal plants. There are many compounds isolated from plants used as conventional medications throughout the world e.g. Taxol from the bark of pacific Yew tree *Taxus brevefolia* for lung disorders, ovarian and breast diseases[8]. *Artemisia annua* containing "artemisinin" utilizes used in the treatment of allergies, respiratory issues, cardiovascular problems, glaucoma, psoriasis, hypothyroidism and weight reduction[9]. Silymarin (mixture of flavonoliganans) extracted from the seeds of *Silybum marianum* (Milk thorn) is used for treating chronic liver diseases, splenomegaly, ascities, hypo-thyroidism, bacterial and viral infections[10].

Interestingly, more than half of the approved Food and Drug Administration (FDA) drugs belong to natural products and their derivatives[11]. Medicinal plants and their parts with phytoconstituents such as alkaloids, tannins, flavonoids and phenolic compounds are widely prescribed in traditional medicine to combat infectious diseases as they possess ability to inhibit the growth and virulence factors of various microbes[12,13]. Resistance to conventional antimicrobial regimes is one of the most serious concerns in global health, and medicinal plants rich in biochemical and bioactive compounds could be a potential source of such new antimicrobial agents[14].

The yellow star thistle or Barnabys thistle, *Centaurea solstitialis* L. (Asteraceae), local to Mediterranean Basin, produces a rosette of non-spiny leaves during the vegetative stage. During summer, its flowering stem produces numerous spinous capitula

* Correspondence:
Phone: +92 3346911256
E-mail: doctor.asif101@gmail.com

containing many (10-50) yellow flowers. It is annual semelparous species and dies once reproduction is completed, usually by the end of summer[15]. *C. solstitialis*, due to its widespread distribution, has common utilization as a remedy in herpes infections around the lips, against peptic ulcer, malaria, common colds, stomach upset and abdominal pain[16]. Despite such valuable efficacy, only a few scientific evaluations of this plant have been documented till now. Therefore, in the present study, we did phytochemical screening of ethanolic extract of *C. solstitialis* and its different fractions, and studied its antioxidant, antibacterial, antifungal and enzyme inhibition activity.

Materials and Methods

Materials and Equipment

All solvents like ethanol (C_2H_5OH), methanol (CH_3OH), n-Hexane (C_6H_{14}), chloroform ($CHCl_3$), ethyl acetate ($C_4H_8O_2$), n-butanol (C_4H_9OH), sulphuric acid (H_2SO_4), perchloric acid ($ClHO_4$) and acetic acid (CH_3COOH) were purchased from Merck, Germany. Vanillin and sodium sulphate anhydrous were purchased from Merck. Ceric (IV), ammonium sulphate was purchased from FlukaChemika.

All glassware including flasks, Buckner funnel, pipettes, beakers were purchased from Pyrex, Japan and Iwaki, Japan. Digital incubator was purchased from Memmert, Germany. Digital electronic weighing balance was purchased from Precisa Instruments, Switzerland. Rotary evaporator 4000 efficient HB digital was bought from Heidolph Laboratory, Germany, whereas recirculating water vacuum pump used with rotary evaporator was bought from VelpScientifica, Europe. Distillation Plant used for distillation of solvents was purchased from Thermo Scientifica, UK. Sonicator was of Elmasonic, Germany. Glass sprayer was supplied by Preciso and Spray flasks were from Witeg, Germany. UV Lamp CAMAG (having wavelengths 254 & 366 nm) were also utilized.

Plant extraction and maceration

Centaurea solstitialis was purchased from Bahawalpur local market and was identified by Dr. Shafiq, Chairman, Department of Botany, Islamia University of Bahawalpur, Pakistan. Two kg of *C. solstitialis* was cut into small pieces and was soaked in four litres of ethanol for 15 days. It was shaken occasionally during the period of maceration. After 15 days, the material was first strained through muslin cloth and then filtered through Buckner funnel. The final amount of filtered extract obtained was 3.8 L. The plant material was again soaked in ethanol for 15 days to ensure complete extraction. The procedure of filtration was again repeated. The filtered extract was 3 L.

Solvent recovery & Qualitative tests

Both filtered extracts were mixed and was subjected to the recovery of solvent in the rotary evaporator at 35°C and 90-120 rpm. Pressure was continuously maintained during the procedure. Concentrated plant extract weighing 800 g was obtained after solvent recovery.

Qualitative phytochemical tests for identification of alkaloids, flavonoids, steroids, glycosides, saponins, tannins and terpenoids were carried out using appropriate methods[17].

Fractionation

Fractionation of the crude extract was done in order to obtain the n-hexane, chloroform, and n-butanol soluble fractions. For this purpose, the crude extract was triturated in 1.0 L water and was divided into three equal parts. Each part was added with double volume of n-hexane in the separating funnel and was shaken occasionally and allowed to stand up to the separation of two layers. Then n-hexane layer was separated. The same aqueous part was passed three times from n-hexane to separate the n-hexane soluble constituents completely. The procedure was repeated for chloroform and n-butanol as well. Final fraction left was water soluble fraction. All the four fractions were dried using rotary evaporator.

Antimicrobial activity

The bacteria used were taken from the Department of Biological and Biomedical Sciences, Aga Khan University Karachi, Pakistan. These bacterial strains were consisted of Gram-positive bacteria which included *Baccilus subtilis* (NCTC 8236), *Staphylococcus aureus* (NCTC 6571) and Gram-negative bacteria which included *Escherichia coli* (NCTC 10418), *Pseudomonas aeruginosa* (ATCC 10145) and *Salmonella typhi*. These bacterial strains were supplied in the form of stock culture agar. The fungal strains *Aspergillus niger* and *Macrophomina phaseolina* were used in the study.

Preparation of culture

Eight grams of nutrient broth (Merck, Germany) was dissolved in 1.0 L of distilled water and then autoclaved at 121°C for 15 min at 15 Psi. Broth was poured in Erlenmeyer flasks and 50 µL of the stock culture bacteria were inoculated in them. These flasks,

containing bacteria were mounted on a horizontal shaker to be shaken at 200 rpm for 24 h at room temperature (25°C). After 24 h turbidity of the culture, growth of the bacteria was measured according to McFarland standard.

96-Well microplate method

Antibacterial activity was performed in sterile 96-well microplates in an aseptic environment. The principle used for this activity is that the bacterial cell number increases in the log phase of the growth proportional to the absorbance[18]. About 20 µg of test sample in methanol was pipetted in each well, and 180 µL of the overnight maintained bacterial culture was poured in each well. Total volume in each well was 200 µL. The pre-read was taken at 540 nm. The plates were incubated at 37°C for 16-24 h. After-read was taken at 540 nm and the difference between pre-read and after-read was taken as an index of bacterial growth. All readings were taken as triplicate. Results are mean of triplicate (n=3, ±SEM). Ciprofloxacin was taken as standard drug.

Minimum inhibitory concentration (MIC)

By making dilutions of test samples, minimum inhibitory concentration was measured by following the same protocol as described above. For calculation of results, EZ-Fit5 Perrella Scientific Inc. Amherst USA software was used.

Agar wells diffusion assay

Muller Hinton Agar was prepared, autoclaved and poured into Petri dishes and was allowed to stand for some time. Ethanolic solutions of the test samples were prepared with a concentration of 10 µg/mL. About 100 µL of the bacterial suspensions were cultivated on agar. With the help of sterile cork borer, holes were made in the hardened agar, and 15 µL of the test solution and the standard solution were poured into the holes with the help of micro pipette. All the Petri plates were incubated for 18-24 h at 37°C. After incubation of 18-24 h, the zones of inhibitions were measured to evaluate the antibacterial activity[19]. The results were obtained by taking average of two experiments. The solvent ethanol did not affect the growth of bacteria. The standard antibacterial used was ciprofloxacin.

Antifungal activity

Eight grams of Sabouraud Dextrose Broth was added in one litre of distilled water. Then it was autoclaved at 121°C at 15 Psi for 15 min. After cooling an antibacterial ciprofloxacin was added into the broth 50 µg/mL of the media to stop the growth of bacteria. The broth was poured in Erlenmeyer flasks and inoculated with stock culture of *Aspergillus niger*[20]. The flasks were mounted on shaker at room temperature for 24 h.

Agar well diffusion method

Sabouraud Dextrose Agar was dissolved in sterilized water and autoclaved at 121°C at 15 Psi for 15 min. Then allowed to solidify and was streaked with the prepared culture of *Aspergillus niger* in four different directions at 90°. With the help of flamed sterile cork borer, bores were made and the prepared test solutions of conc. 0.1 mg/mL were poured into the bores. Standard used was fluconazole in concentration of 10 µg/mL. A control having DMSO was also included in each plate. The Petri plates were kept inverted to allow the diffusion of the test solution for one hour, and then the plates were incubated at 25°C. After 24-36 h zone of inhibition was measured[20]. The same procedure was performed three times and the results are the mean of three independent readings.

Antioxidant activity by DPPH free radical scavenging assay

Antioxidant activity by DPPH free radical scavenging assay of *C. solstitialis* was determined[21]. Methanol was used to prepare different concentrations of test solutions. About 100 µM DPPH solution was prepared in methanol, and in a 96-well plate, 10 µL of test solution and 90 µL of DPPH solution were added. The contents were mixed and incubated at 37°C for 30 min. The reduction in absorbance was measured at 517 nm using Synergy microplate reader (HT BioTek® USA). Standard antioxidant used was quercetin. All experiments were carried out in triplicate. IC_{50} values which are required to scavenge 50% of DPPH were computed using Amherst USA software Ez-fit5 Perrella Scientific Inc.[22].

Enzyme inhibition assays

Acetylcholinesterase and butrylcholinesterase inhibition assays of *C. solstitialis* were performed according to the method described by Orhan *et al*.[23] with slight modifications. About 10 µL of test compound 0.5 mM $well^{-1}$, 60 µL Na_2HPO_4 buffer 50 mM and pH 7.7 and 10 µL (0.5 mM $well^{-1}$) acetylcholinesterase or butyrylcholinesterase enzyme was added in a 96-well plate. The components of the assay were mixed and pre-read was taken at 405 nm before subjected to incubator at 37°C for 10 min. The reaction started when 10µL of 0.5 mM $well^{-1}$ substrate (butyrylthiocholine chloride) was incorporated followed by pouring of 10 µL DTNB 0.5 mM $well^{-1}$. It

was placed in incubator for 30 min at 37°C, and reading was taken at 405 nm using 96-well plate reader (Synergy HT, Biotek, USA). Eserine was used as standard. All readings were taken in triplet.

Chymotrypsin assay of *C. solstitialis* was also performed. The reaction mixture in the cuvette of the spectrophotometer consisted 0.3 mL of a solution of chymotrypsin (333 pg/mL) in 0.004 M acetic acid and 0.02 M $CaCl_2$ plus 0.0-0.7 mL of a solution containing 150 pg of inhibitor per mL in 0.006 M Tris buffer at pH 8.2, and 0.7 to 0.0 mL of Tris buffer to bring the volume to 1.0 mL. To this were added 2 mL of substrate-buffer-indicator solution (0.008 M BTE, 30% methanol, 0.0075 M Tris-HCl buffer, and 0.01% nitrophenol with a final pH value of 8.2)[4].

These assay contents were mixed well and pre-read was taken at 234 nm and incubated for 10 min at 25°C. The reaction was initiated by adding 25 µL substrate solution. The changes in absorbance were measured after 6-10 min at 234 nm. PMSF was used as a standard. All readings were taken in triplicate and results were taken as mean of three independent experiments (mean±SEM). IC_{50} values were determined by assaying the test solution at various dilutions i.e. 0.5, 0.25, 0.125, 0.0625, 0.0313, 0.015 mg. The results were computed on Amherst USA software, Ez-fit5 Perrella Scientiic Inc.

Statistical analysis

Data were expressed as mean±S.D. of three replicates and analyzed by SPSS version 19. Tukey Post-Hoc one-way ANOVA was used for determination of differences among mean. Values of P <0.05 were regarded as significant.

Results

Phytochemical analysis

The phytochemical analysis of ethanolic extract of *Centaurea solstitialis* showed the presence of alkaloids, terpenoids, gums, flavonoids, glycosides, tannins and reducing sugars.

Antibacterial activity

Significant (P <0.05) antibacterial activity was observed by ethanolic extract of *C. solstitialis* and all its fraction against *S. typhi*, *E. coli*, *B. subtilis*, *S. aureus* and *P. aeruginosa* when compared to the standard ciprofloxacin evaluated through 96-well microplate method (Table 1) and agar well diffusion method (Fig. 1).

Antifungal activity

Significant (P <0.05) antifungal activity of ethanolic extract of *C. solstitialis* and its different fractions was observed against *A. niger* and *M. phaseolina* comparable with that of standard fluconazole (1.0 mg/mL). The results are given in Fig. 2.

Antioxidant activity (DPPH assay

The antioxidant potential of *C. solstitialis* and all its fractions by DPPH assay were found to be significant (P <0.05) when compared to the standard quercetin.

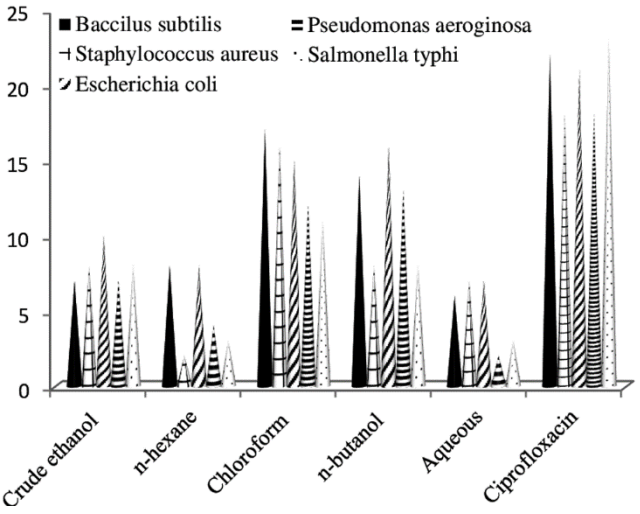

Fig. 1 — Antibacterial activity of *Centaurea solstitialis* by agar well diffusion method against five human pathogens; *B. subtilis* (+), *S. aerus* (+), *E. coli* (–), *P. aeroginosa* (–) and *S. typhi* (–)

Table 1 — Antibacterial activity of *Centaurea solstitialis* by 96-wells Microplate method

Samples	*Salmonella typhi*		*E. coli*		*Bacillis subtilis*		*Staphylococcus aureus*		*P. aeroginosa*	
	% inhibition	MIC (µg/mL)	% inhibition	MIC (µg/mL)	% inhibition	MIC (µg/mL)	% inhibition	MIC (µg/mL)	% inhibition	MIC (µg/mL)
Crude ethanol	69.37±2.11^a	9.34±0.31	76.53±0.07^a	8.62±0.00	70.43±0.97^a	9.78±0.23	52.20±0.10	17.04±0.38	69.07±0.47^a	10.00±0.21
n-hexane	75.53±0.97^b	8.79±0.65	74.10±0.40^b	9.05±0.24	51.30±2.00^b	17.69±0.31	52.97±0.57	17.65±0.07	71.43±1.27^b	9.51±0.90
Chloroform	51.90±0.60^c	17.96±0.12	66.47±0.37^c	10.20±0.02	53.73±2.07^c	16.57±0.81	46.33±1.17	-	54.40±0.10^c	16.72±0.56
n-butanol	53.83±1.83^d	16.48±0.33	56.33±0.73^d	16.21±0.36	57.50±0.00^d	14.53±0.13	42.43±0.57	-	57.53±0.47^d	15.78±0.58
Aqueous	78.13±0.33^e	8.55±0.14	69.60±0.90^e	9.39±0.34	77.10±1.00^e	8.53±0.85	57.20±1.00	16.23±0.89	75.43±0.87^e	9.25±0.16
Ciprofloxacin	90.10±0.40	7.46±0.09	92.83±0.13	7.14±0.37	91.43±0.27	7.21±0.79	90.47±0.07	7.63±0.68	91.77±0.13	7.28±0.68

[Values are mean (n=3), ± standard deviation, superscript a, b, c, d and e shows that mean± standard deviation in the same column with different superscript are significantly different (P <0.05)]

Only n-hexane soluble fraction showed insignificant result (Table 2).

Enzymes inhibitory essays

The results of acetylcholinesterase (AChE), butyryl-cholinesterase (BChE) and chymotrypsin (CHYM) enzyme inhibition assays are showing that n-butanol fraction exhibited significant acetylcholinesterase and butrylcholinesterase inhibitory activity (78.55±0.76 %) with IC_{50} value of 54.6±0.39 µg/mL and (78.1±0.41%) with IC_{50} value of 211.9±0.15 µg/mL, respectively. Maximum chymotrypsin inhibition activity was shown by crude ethanolic extract (87.76±1.17) with IC_{50} value of 38.23±0.75 µg/mL. Details are given in Table 3.

Discussion

The genus *Centaurea* has known therapeutic potential and has assumed huge interest in the discovery and design of novel drug formulations[25]. In the present work, the results of free radical scavenger activity of *Centaurea solstitialis* crude ethanolic extract and its different fractions showed significant DPPH scavenging activity. The results of all the fractions were found to be significant (P <0.05) when compared to standard quercetin. Only n-hexane soluble fraction showed insignificant result. Antioxidant-based formulations are used for the prevention and treatment of several diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer[26]. Natural antioxidants that are present in herbs and spices are responsible for inhibiting or preventing the adverse effects of oxidative stress due to the presence of free radical scavengers like polyphenols, flavonoids and phenolic compounds[27].

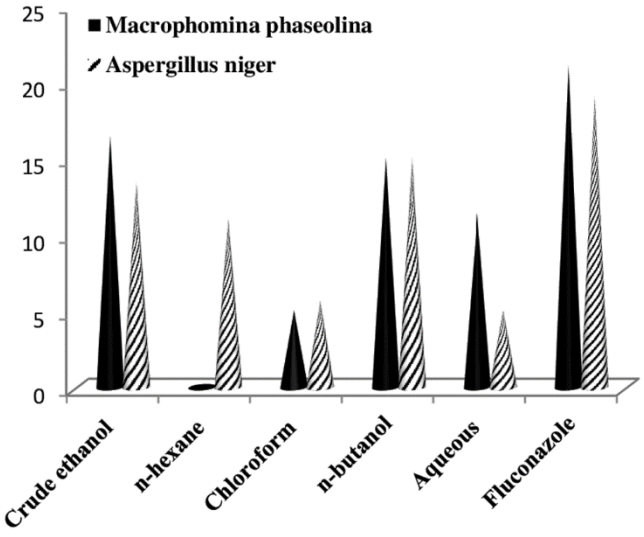

Fig. 2 — Antifungal activity of ethanolic extract of *Centaurea solstitialis* and its different fractions against *Aspergillus niger* and *Macrophomina phaseolina*

Significant acetylcholinesterase (AChE), butyryl-cholinesterase (BChE) and chymotrypsin inhibitory activity of *C. solstitialis* was observed in our study and the results are comparable with previous reports[28]. Hence, it is evident that *C. solstitialis* can be used as a potent source of natural AChE and BChE inhibitors. It can be used as nervine stimulant, lowering the risk of Alzheimer's disease which is a neurological disorder leading to behavioural disturbances, cognitive dysfunction and memory impairment[29].

Crude ethanolic extract of *C. solstitialis* and its different fractions were investigated for their antibacterial activity by agar well diffusion method against five human pathogens *S. typhi* (–), *E. coli* (–), *B. subtilis* (+), *S. aerus* (+), *P. aeruginosa* (–). Results showed that significant antibacterial activity was shown by chloroform soluble fraction (17 mm) against *B. subtilis*, (16 mm) against *S. aureus* and (17 mm) against *E. coli*. n-Butanol soluble fraction of

Table 2 — Antioxidant activity of *Centaurea solstitialis* by DPPH Assay

Fraction used	Conc. (µg/mL)	DPPH value % inhibition
Crude ethanolic extract	0.5	88.52±0.23[a]
n-hexane soluble fraction	0.5	36.73±1.45[b]
Chloroform soluble fraction	0.5	69.23±2.76[c]
n-butanol soluble fraction	0.5	88.33±1.32[d]
Aqueous fraction	0.5	85.84±0.78[e]
Quercetin (mmol/mL)	0.05	93.74±0.12[f]

[Values are mean (n=3) ± standard deviation. Superscript a,b,c,d,e and f showed that mean± standard deviation in the same column with different superscript are significantly different (P <0.05)]

Table 3 — Enzymes inhibition activity of *Centaurea solstitialis*

Fractions	Conc. (mg/mL)	AchE % Inh	IC_{50} µg/mL	BChE %Inh	IC_{50} µg/mL	CHYM %Inh	IC_{50} µg/mL
Crude ethanol	1.0	70.83±0.97	135.1±0.59	42.3± 0.14[a]	-	87.76±1.17[a]	38.23±0.75
n-hexane	1.0	–45.10±0.12	-	11.2±0.58[b]	-	83.55±1.11[b]	62.49±0.98
Chloroform	1.0	–47.99±0.46	-	40.7± 0.32[c]	-	63.23±1.14[c]	20.51±0.98
n-butanol	1.0	78.55±0.76	54.6±0.39	78.1± 0.41[d]	211.9±0.15	39.92±1.15[d]	-
Eserine	0.25*	92.25±1.18	0.04±0.0001***	92.25±1.18[e]	0.04±0.0001***	-	-
PMSF	0.5**	-	-	-	-	96.71±0.79[e]	48.71±0.13[#]

[*mMol/L; ** mMol/mL; *** µMol/L; and [#] µMol/mL. Values are mean (n=3) ± SD. Superscript a, b, c, d and e shows that mean ± SD in the same column with different superscript are significantly different (P <0.05)]

C. solstitialis inhibited *E. coli* growth by 16 mm, *B. subtilis* by 14 mm and *P. aeruginosa* by 13 mm. Crude ethanolic extract and n-hexane soluble fraction also showed significant activity against pathogenic bacteria. Moreover, our result indicated that n-butanol soluble fraction has significant antifungal activity against *A. niger*. All other fractions also inhibited the growth of *A. niger*.

The results of our study are comparable with a previous report on biological activities of two species of Genus *Centaurea*, namely *C. drabifolia* and *C. lycopifolia* in which authors evaluated the antimicrobial effects against 16 bacterial and fungal strains using microdilution method as well as antioxidant potential was evaluated by DPPH and ABTS radical scavenging assays[30]. In another report, authors evaluated the possible enzyme inhibitory potential, antioxidant activity, and phytochemical profile of *C. saligna* in which ethyl acetate extract showed strong antioxidant ability and inhibitory potential against cholinesterase, butyryl cholinesterase and α-glucosidase enzymes[31]. Five flavonoids *viz*., hispidulin, cirsimaritin, apigenin, apigenin 7-O-glucoside and isokaempferide isolated from *Centaurea nerimaniae*, have been shown to inhibit AChE and lipid peroxidation, scavenge DPPH radicals and antimicrobial potentia Nikolova & Bancheva[33] have reported that the aerial parts of *Centaurea* species is a potent source of phenolic compounds. *C. davidovii* and *C. parilicaare* possess flavonoid aglycones such as apigenin, luteolin, kaempferol 3-methyl and scutellarein 6,4'-dimethyl ethers and 6-hydroxyluteolin 6-methyl and could act as an anti-inflammatory and antioxidant agent[33].

Irrational and inappropriate uses of antibiotics have resulted in the antimicrobial resistance leading to an increased interest in medicinal plants for the discovery of new antibiotics[34]. Approximately, 30-50% of recent nutraceuticals and pharmaceuticals are derived from plants[35]. Secondary metabolites and phytochemicals in the plants possess great value to act as therapeutics[36]. It is estimated that by 2050, the mortality ratio due to antimicrobial resistance will inflatable to 10 million lives annually at an expense of one hundred trillion dollars[37].

Plant oriented antimicrobial agents act usually through the lysis of the cell membranes and cell wall of microbes, which ultimately lead to the release of cellular content, enzyme inactivation, protein binding domain disruption and finally death of the cells[38]. Furthermore, antioxidant and enzyme inhibitory properties of medicinal plants will lead to the solution of various health problems including diabetes, cancer, cardiovascular diseases, atherosclerosis, memory deficit, Alzheimer's disease and many others[39]. The antimicrobial, antioxidant and enzyme inhibitory results of *C. solstitialis* in our study are comparable to the findings of the above discussed studies as well as many other reports[40-42]. These studies support the therapeutic potential of *C. solstitialis* against different diseases.

Conclusion

Centaurea solstitialis crude ethanolic extracts and its different fractions exhibited significant antibacterial (*S. typhi*, *E. coli*, *B. subtilis*, *S. aureus* and *P. aeruginosa*) and antifungal (*A. niger* and *M. phaseolina*) activities apart from significant DPPH scavenging activity among all fractions. The n-butanol fraction showed significant acetylcholinesterase inhibitory activity (78.55±0.76%) and butrylcholinesterase inhibitory activity (78.1±0.41%) with IC_{50} values of 54.6±0.39 and 211.9±0.15 µg/mL, respectively. Maximum chymotrypsin inhibition activity was shown by crude ethanolic extract (87.76±1.17) with IC_{50} value of 38.23±0.75 µg/mL. These biological activities of *C. solstitialis* could be due to its phytochemical contents, such as alkaloids, terpenoids, gums, flavonoids, glycosides, tannins and reducing sugars present in the plant extracts.

Conflict of interest

Authors declare no conflict of interests.

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