OPEN ACCESS

Phytopharmacological Communications

DOI: doi.org/10.55627/ppc.003.002.0434

Research Article

Chemical Composition, Antioxidant, and Anticancer Potential of *Anchusa arvensis*

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Abstract

The purpose of this study was to investigate the phytochemical composition, antioxidant characteristics, and cytotoxic effects of *Anchusa arvensis (A. arvensis)* crude extract (Aa.Cr) and its subsequent fractions: *n*-hexane (Aa.Hex), chloroform (Aa.Chm), ethyl acetate (Aa.Et), and aqueous (Aa.Aq). The antioxidant potential was determined using the diphenyl-1-picrylhydrazyl (DPPH) and azinobis [3-ethylbenzthiazoline]-6-sulfonic acid (ABTS) radical scavenging assays. The sulforhodamine-B assay demonstrated anticancer activity against the human cancer cell line NCI-H460. Moreover, alkaloids, saponins, flavonoids, glycosides, and tannins were found in Aa.Cr. The Aa.Chm fraction showed the highest DPPH radical scavenging activity (74.83 ± 1.36%) at 1000 µg/mL concentration. The extract fractions Aa.Hex and Aa.Et demonstrated moderate activity, while Aa.Aq showed poor radical scavenging activity. The results show that the Aa. Hex has significant ABTS radical scavenging activity with IC_{50} of 500µg/mL. Remarkable cytotoxicity was observed for Aa.Chm fraction with a growth inhibitory (GI_{50}) value of 31 ± 0.6µg/mL, followed by an Aa.Hex GI_{50} value of 45 ± 5.7µg/mL. On the other hand, Aa.Aq, and Aa.Et fractions failed to exhibit growth inhibitory effects against NCI-H460 cells. These findings indicate that Aa.Cr and its derived fractions possess antioxidant and anticancer effects.

Keywords: *Anchusa arvensis*, phytochemical, antioxidant, anticancer, medicinal plants, phytocompounds

1. Introduction

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) have the ability to oxidize lipids, and proteins and damage DNA in biological systems. The antioxidant system of the human body can scavenge free radicals, preserving the equilibrium between oxidation and anti-oxidation. Nonetheless, excessive ROS and RNS produced by cigarette smoking, alcohol, radiation, and environmental pollutants alter the balance of oxidants and antioxidants, leading to a variety of chronic and degenerative disorders. Moreover, exogenous antioxidants block the onset or propagation of oxidative chain reactions by acting as free radical scavengers, singlet oxygen quenchers, and reducing agents (Lee et al. 1998, Krishnaiah et al. 2011). Oxidative stress is a foremost cause of illness in humans as free radicals and ROS attack cell macromolecules. Therefore, antioxidants play a critical role in preventing ROS-induced cell damage by counter-acting free radicals (Atawodi 2005, Rajeshkumar et al. 2019, Alam et al. 2022)

Several of the clinically available drugs are derived from medicinal plants. For example, over 60% of cancer treatments are derived from nature, including plants like vincristine and vinblastine (Moudi et al. 2013, Amin et al. 2009, Kinghorn et

al. 2003). Paclitaxel, isolated from, *Taxus brevifolia* plant is used in the treatment of breast, ovarian, and non-small lung cancer. Similarly, teniposide and camptothecin derivatives are also obtained from natural sources (Pezzuto 1997, Iqbal et al. 2017). The Boraginaceae family includes several genera; *Anchusa* is an important genus among them. Several *Anchusa* species possess medicinal properties including wound healing, and anti-gout activities (Al-Snafi and Sciences 2014). It also contains anticancer, antioxidant, and antiviral activities, and affects the central nervous system, and endocrine systems. *Anchusa* has been proven to have gastro-protective, antimicrobial, hypotensive, and anti-diabetic activities (Khomsi et al. 2022, Selvi and Bigazzi 2003). The antibacterial and anticancer potential of *A. arvensis* has also been reported (Hussain, Ullah, Ayaz, et al. 2019, Hussain, Ullah, Sadiq, et al. 2019). The current study was designed to explore the presence of secondary metabolites for possible antioxidant and anticancer effects, based on its traditional medicinal usage.

Table 1. The phytochemical components of the *A. arvensis* crude extract (Aa.Cr)

Phytochemical class	Result
Alkaloids	**positive**
Saponins	**positive**
Flavonoids	**positive**
Glycosides	**positive**
Cardiac glycosides	**positive**
Tannins	**positive**
Terpenoids	**positive**
Phenolic Compounds	**positive**
Steroids	**positive**

2. Methods & Materials

2.1. Drugs and Chemicals

Dimethyl sulphoxide (DMSO), DPPH (diphenyl-2-picrylhydrazyl) ABTS, (azinobis[3-ethylbenzthiazoline]-6-sulfonic acid). Doxorubicin, sulforhodamine-B, ascorbic acid, acetic acid, methanol, chloroform, ethyl acetate, and *n*-hexane were purchased from Sigma Aldrich Chemicals (Sigma Chemicals Co., St. Louis, MO, USA).

2.2. Plant Collection and Preparation of Extracts

The *A. arvensis* was obtained from distract Karak in Khyber Pakhtunkhwa, Pakistan. The botanical identity was confirmed by Dr. Waheed Murad, Department of Botany, Kohat University of Science and Technology. A voucher specimen with the catalog number (KUH-1001) was submitted to the department's herbarium. The plant was crushed to fine powder and extracted for 20 days at room temperature with periodic shaking (Khan et al. 2013). The methanolic extract was filtered and concentrated at a low temperature (40°C) using a rotary evaporator. The filter yielded a crude extract (600 g). The crude methanol extract (500 g) was suspended in distilled water (500 ml) and fractionated with *n*-hexane, chloroform, and ethyl acetate, yielding Aa.Aq (60gm), Aa.Hex, (135gm) Aa.Chm (160gm), and Aa.Et (90gm) fractions, respectively. These fractions were then tested for selected pharmacological activities.

2.3. Preliminary Phytochemical Screenings

Preliminary phytochemical investigation of the plant crude extract was performed by using standard procedures, with slight modifications (Trease and Evans 1989, Harborne 1998).

Table 2. Antioxidant activity of *A. arvensis* extracts against DPPH.

Sample	Concentrations (µg/ml)				IC_{50} (µg/mL)
Plant extracts	125	250	500	1000	-
Aa.Cr	45.00 ± 0.57***	51.66 ± 1.76***	63.16 ± 1.01***	67.00 ± 1.76**	200
Aa.Hex	37.00 ± 1.15***	42.66 ± 2.33***	47.33 ± 0.88***	54.66 ± 1.20***	640
Aa.Chm	52.66 ± 0.88***	62.00 ± 1.52***	66.00 ± 1.50**	74.83 ± 1.36*	60
Aa.Et	39.00 ± 1.15***	43.00 ± 2.30***	51.33 ± 1.02***	54.66 ± 2.02***	520
Aa.Aq	36.00 ± 1.73***	41.66 ± 1.76***	46.00 ± 1.15***	51.33 ± 2.02***	850
Ascorbic acid	74.66 ± 1.51	77.00 ± 0.00	81.33 ± 2.12	85.00 ± 1.00	5

The data is the average standard error of three separate readings (n = 3). Values expressed as Percent inhibition (Mean ± SEM of n = 3) and IC_{50}.

2.4. DPPH Radical Scavenging Assay

This activity was carried out according to the established method with slight changes (Ayaz et al. 2014, Braca et al. 2001). DPPH solution was prepared by dissolving 24 mg of it in 100 mL of methanol. Stock solutions of test samples (1 mg/mL) were made in methanol, followed by serial dilution in concentrations ranging from 125-1000µg/mL. After thirty minutes, the absorbance at 517 nm was measured with a spectrophotometer. The percentage blockage of radical scavenging capacity was calculated by comparing the results to the control, ascorbic acid served as a positive control. The entire analysis was done in triplicate. Lower absorbance of the reaction mixture indicated high free radical scavenging activity. The IC_{50} values were calculated with the Graph Pad Prism program (Graph PAD, San Diego, California, USA). The ability to scavenge the DPPH radical was calculated using the following equation:

$$\textit{DPPH scavenging effect (\%)} = [(A_1 - A_2)/A_1] \times 100$$

Where A1 is the absorbance of the control reaction, and A2 is the absorbance in the presence of the sample.

2.5. ABTS Free Radical Scavenging Assay

The antioxidant potential of different plant fractions was determined using the ABTS scavenging protocol (Re et al. 1999). The radical was dissolved in deionized water to a concentration of 7 mM before mixing with 2.45 mM potassium persulphate. The reaction mixture was allowed to stand at room temperature for 12 to 16 hours in the dark prior to usage. The resulting highly colored ABTS radical cation was diluted with 0.01 M PBS, with pH 7.4, yielding an absorbance value of 0.70 at 734 nm. After each extract was added, absorbance was measured spectrophotometrically, at 1-minute intervals. Whereas, ascorbic acid served as the positive control, and the percent inhibition was calculated using the following formula:

$$\textit{ABTS Scavenging Effect (\%)} = [\textit{control absorbance} - \textit{sample absorbance} / \textit{control absorbance}] \times 100$$

Table 3. Antioxidant activity of plant extracts of *A. arveniss* against ABTS.

Sample	Concentrations (µg/ml)				IC50 (µg/mL)
Plant extracts	125	250	500	1000	-
Aa.Cr	24.33 ± 0.88***	39.33 ± 0.72***	42.66 ± 0.98***	52.00 ± 1.24***	900
Aa.Hex	17.66 ± 1.44***	23.00 ± 1.00***	37.66 ± 2.08***	61.50 ± 0.50***	500
Aa.Chm	31.66 ± 0.33***	35.00 ± 1.50***	41.00 ± 0.57***	53.68 ± 3.52***	810
Aa.Et	26.50 ± 0.57***	31.33 ± 0.88***	35.00 ± 0.00***	45.35 ± 1.65***	1220
Aa.Aq	29.50 ± 1.50***	31.00 ± 1.15***	39.25 ± 1.66***	46.00 ± 0.00***	1100
Ascorbic acid	69.33 ± 0.66	73.68 ± 3.52	77.32 ± 1.32	81.65 ± 1.65	<5

Values significantly differ as compared to positive control. Values expressed as Percent inhibition (Mean ± SEM of n = 3) and IC_{50}.

The antioxidant effect was expressed in terms of percent inhibition and as IC_{50}.

2.6. Anti-Cancer Activity

The sulforhodamine-B assay was used to assess the growth inhibitory activity of methanol extract and its resultant fractions against the human cancer cell line NCI-H460 (non-small cell lung carcinoma) (Skehan et al. 1990). Doxorubicin was utilized as a control medication. The cells (10,000 cells/100µL) were placed in each well of a 96-well plate and incubated at 37°C in a humidified 5% CO_2 incubator for 24 hours. Stock solutions of Aa.Cr and fractions (40 mg/mL) and doxorubicin (1 mM) were prepared in DMSO. Various dilutions of methanolic extract and fraction (10, 50, 100, 200, and 250µg/mL), doxorubicin (0.1, 0.25, 0.5, 0.75, 1.0µM) were added (100µL) in each well. After 48 hours of incubation, cold (4°C) trichloroacetic acid (50%, 50µL) was smoothly added, and left for 30 minutes at room temperature before rinsing with distilled water, followed by drying overnight at room temperature. After 10 minutes, 100µL of sulforhodamine B solution (0.4% w/v in 1% acetic acid) was incorporated into each well, and 1% acetic acid was used to wipe away the free stain. The dye was dissolved in tris-base (pH 10.2) for five minutes, and a microplate reader was used to detect the dye at 515 nm.

2.7. Statistical Analysis

The one-way analysis of variance (ANOVA) technique was used for the calculation of SEM followed by Duncan's multiple range using the SPSS 17 program. Differences less than 0.05 were considered significant.

3. Results

3.1. Phytochemical Analysis

Different Fractions, Aa.Cr tested positive for the presence of important secondary metabolites **(Table 1).**

3.2. DPPH Radical Scavenging Ability

Overall, the chloroform portion of the plant was found to be more effective by scavenging DPPH radicals (IC_{50} = 60 µg/ml) while Aa.Aq presented weak radical scavenging activity (IC_{50} = 850 µg/ml) **(Table 2)**.

3.3. ABTS Radical Scavenging Ability

Aa.Cr, and its derived fractions, exhibited radical scavenging activity against ABTS in a

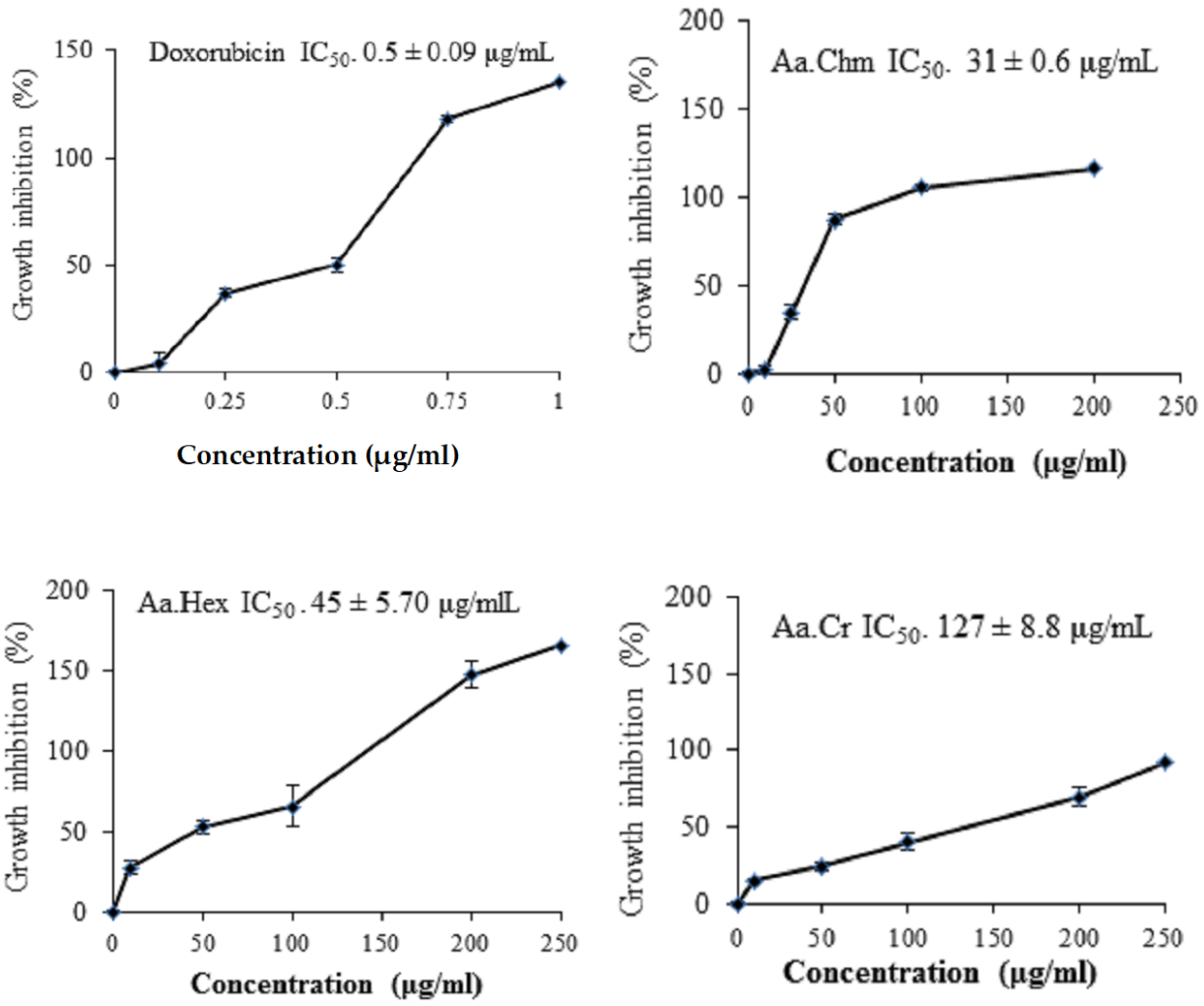

Figure 1: Concentration-dependent anticancer effects of crude extract of A. arvensis (Aa.Cr) and its fractions.

concentration-dependent manner. Furthermore, results indicated that the Aa.Hex has significant ABTS radical scavenging activity of 17.66 ± 1.44 %, 23.00 ± 1.00 %, 37.66 ± 2.08 %, and 61.50 ± 0.50 % at the dose of 125, 250, 500, and 100µg/mL, respectively, with IC_{50} of 500µg/mL. Moreover, Aa.Chm at the dose of 125, 250, 500, and 1000 µg/mL showed ABTS radical scavenging activities of 31.66 ± 0.33 %, 35.00 ± 1.50 %, 41.00 ± 0.57 % and 53.68 ± 3.52 respectively with an IC_{50} of 810µg/mL. ABTS radical scavenging activities of Aa.Cr at the dose of 125, 250, 500, and 100 µg/mL was 24.33 ± 0.88, 39.33 ± 0.72, 42.66 ± 0.98, and 52.00 ± 1.24 µg/mL respectively with IC_{50} value of 900µg/mL. While IC_{50} value of Aa.Aq, and Aa.Et was 1100 and 1220 µg/mL, respectively **(Table 3)**.

3.4. Anticancer Effects Against NCI-H460 Cancer Cells

Aa.Cr was found to be highly cytotoxic against NCI-H460 cancer cells in a dose-dependent manner (50-250 µg/mL) with a GI_{50} value of 31 ± 0.6 µg/mL, as shown in *Figure 1*. The hexane fraction of the Aa.Cr also inhibited cancer cell growth at the dose of (50-250 µg/mL) with GI_{50} value of 45 ± 5.70µg/mL. However, this effect was less potent than that of Aa.Chm extract. Marginal growth inhibition of cancer cells at a dose (50-250 µg/mL) was induced by Aa.Cr, with a GI_{50} value of 127 ± 8.8µg/mL but it was 4 and 3 folds less

potent than that of Aa.Chm and Aa.Hex extracts of Aa.Cr respectively. In contrast, the Aa.Aq and Aa.Et fractions of Aa.Cr did not cause growth inhibitory effects at the maximum dose of 250 μg/mL against NCI-H460 cells, while the IC_{50} value of doxorubicin used as a positive control was 0.5 ± 0.09μg/mL

4. Discussion

Plants are a rich source of secondary metabolites with biological activity that could be used to treat a wide range of diseases (Pagare et al. 2015). Natural products are safe, affordable, and widely accessible. However, phytochemical screenings, isolations, and pharmacological research are crucial elements for the development of innovative phytomedical cures. In our study, the Aa.Cr fraction revealed the presence of medicinally active compounds, like alkaloids, saponins, flavonoids, glycosides, cardiac glycosides, tannins, terpenoids, phenolic compounds, and steroids. Many plants have been studied for their antioxidant activity as free radicals are known to be responsible for a wide range of clinical symptoms. Antioxidants are used to treat a variety of chronic diseases by scavenging reactive oxygen species (Pagare et al. 2015). DPPH is a frequently used model to assess the scavenging capacity of a variety of natural chemicals. The DPPH radical scavenging experiment was performed on various fractions of *A. arvensis* during which the Aa.Chm showed significant radical scavenging capacity **(Table 2)**. The radical scavenging activity of Aa.Cr, and its subsequent fractions, were found to be concentration-dependent. The Aa.Hex and Aa.Chm fractions demonstrated significant ABTS radical scavenging activity with IC_{50} of 500 and 810μg/ml, respectively. According to the findings of this study, the plant has phytochemical constituents that may contribute to its antioxidant capacity. Tannins and phenols have been identified as free scavenging agents (Figueroa-Espinoza et al. 2015, Maqsood et al. 2014). Plant-derived flavonoids and saponins have a well-established antioxidant capacity (Xi et al. 2010, Hong et al. 2014).

Among different fractions, the Aa.Chm fraction exhibited significant cytotoxicity activity against NCI-H460 cells (GI_{50} 31 ± 0.6 μg /m) followed by Aa.Hex (GI_{50} 45 ± 5.7). The Aa.Cr fraction of *A. arvensis* demonstrated cytotoxicity with GI_{50} of 127 ± 8.8 μg /ml. The Aa.Aq and Aa.Et fractions did not exhibit an inhibitory effect against NCI-H460 cells. Previously, three new rotenoids and two new coumaronochromonoids, as well as 10 known compounds from the medicinal plant *Boerhaavia erecta* were tested for cytotoxicity against the HeLa, NCI-H460, and MCF-7 cell lines. (Do et al. 2013). Similarly, *Streptocaulon juventas* cardiac glycosides demonstrated significant inhibitory effects against human lung A549 adenocarcinoma cells, lung cancer cell lines, and normal human fetal lung fibroblast MRC-5 cells. (Xue et al. 2014). Moreover, a potent anti-proliferative activity of compounds, isolated from *Saururus chinensis,* against NCI-H460 was reported (Lee et al. 2012). Similarly, different plant extracts were found to have anticancer properties against various cancer cell lines in Brazil. (Suffredini et al. 2007). Cytotoxicity of three compounds isolated from *Withania somnifera* against a human lung cancer cell line was also reported (Choudhary et al. 2010). Anti-proliferative activity was demonstrated by methoxylated flavonoids isolated from leaves of *Zeyheria montana Mart*, against human tumor cell lines UACC-62), MCF-7, 786-0, NCI-ADR/RES, lung non-small cells, PC-3 , OVCAR-3, HT-29, and K562 cancer cell lines (Seito et al. 2011). The present study add to the relevant literature by showing that Aa.Chm fraction can be a safe source of antioxidant and cytotoxic agents against the NCI-H460 cell line. A bio-guided fractionation performed on this fraction would be helpful to elucidate the active cytotoxic agent and antioxidant agents.

5. Conclusions

This is the first research on the pharmacological and phytochemical characteristics of *A. arvensis*

extracts. Our findings demonstrated that Aa.Chm fraction may be used to isolate antioxidant and cytotoxic substances that are harmful to the NCI-H460 cell line. Further in-depth molecular studies are warranted to investigate the nature of chemical constituents and confirm the pharmacodynamic basis of biological activities.

Conflict of Interest

The authors declare that they have no competing interests.

Funding

NA.

Study Approval

The study was approved by the Department of Pharmacy, Kohat University of Science & Technology, Kohat, Pakistan.

Consent Forms

NA.

Authors Contribution

SH performed experimental work, data collection and evaluation, literature search, and manuscript preparation. FA, TH, SA, HHB, and SMH supervised the research work and refined the manuscript for publication. The authors read and approved the final manuscript for publication.

Data Availability

All the relevant data of this manuscript is available with the authors.

Acknowledgments

The authors are thankful to the Department of Pharmacy, Kohat University of Science & Technology, Kohat, Pakistan.

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