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Phytochemical study on the constituents from *Cirsium arvense*

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Abstract: Phytochemical investigation on the chloroform soluble fraction of *Cirsium arvense* resulted in the isolation of five compounds namely *Ciryneol C, Scopoletin*, *Pectolinarigenin-7-O-glucopyranoside*, *Acacetin and 6, 7-Dimethoxycoumarin*. Their structures have been elucidated by EIMS, HREIMS, ^{1}H and ^{13}C NMR spectroscopic methods. These compounds have been isolated for the first time from this plant. All the isolated compounds were tested for their antibacterial and antifungal activities.

Keywords: *Cirsium arvense*, Fractionation, Isolation, Antibacterial and Antifungal activities.

Introduction

Circum arvence is a medicinal plant belonging to family *Asteraceae*[1] and is often found as noxious weed in grasslands and riparian habitats[2]. *C. arvense* is known to reduce forage biomass[3] as well as its favourable response to fertilization[4,5]. A recent study shows that the foliar endophytic fungal community composition in *Cirsium arvense* is affected by mycorrhizal colonization and soil nutrient content [6].

The genus *Circum* is popular for an array of medicinal uses such as in treatment of peptic ulcer and leukaemia in folk medicine[7]. It has been used in cure of epitasis, metrorrhagia, syphilis [8] eye infections, skin sores gonorrhoea, bleeding piles and has also been found to be effective against diabetes[9,10]. American Indians purportedly used an infusion of *C. arvense* roots for mouth diseases, worms and poison-ivy (*Toxicodendron radicans*) and in treatment for tuberculosis[11]. *C. arvence* roots are reported to contain arsenic-resistant bacteria[12]. A recent study revealed that Nonenolides and cytochalasins exhibited strong phytotoxic activity against *C. arvense* leaves[13]. Fragrance of C. *arvense* attracts both floral herbivores and pollinators[14].

Cirsium arvense is found in very considerable quantities in District Bannu, Pakistan and locally known as "*Aghzikai"*. It has been used medicinally in different areas from very beginning till now. It is known to be diuretic, astringent, anti-phlogerstic and hepatic[8] mainly due to existence of various flavonoids and coumarins[15]. Previous studies suggested that Flavonoid compounds, phenolic acids, tannins, sterols and triterpenes are the main constituents of genus *Cirsium*[16-19]. The methanolic extract and $CHCl_3$, Et_2O, EtOAc, *n*-BuOH fractions of *C. arvence* possesses highly significant antioxidant activity and total phenolic contents[20].

No work has been reported so far on this species. The diverse medicinal uses attributed to this species prompt us to carry out phytochemical investigation and biological activities on this plant.

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The methanolic extract of the whole plant of *C. arvense* showed strong toxicity in *brine shrimp lethality* test[21]. On further fractionation, the maximum toxicity was observed in chloroform and ethyl acetate soluble fractions. As a result of a series of chromatographic resolutions of the chloroform soluble fraction, five compounds were isolated from this species namely *ciryneol C* **1**, *scopoletin* **2**, *pectolinarigenin-7-O-glucopyranoside* **3**, *acacetin* **4** and 6,7-Dimethoxycoumarin **5** respectively. Their structures were confirmed on the basis of spectral data reported in the literature. All of these compounds were tested for their antibacterial activity and antifungal activities.

Results and Discussion

The methanolic extract was fractionated into *n*-hexane, $CHCl_3$, EtOAc and *n*-BuOH soluble fractions. The repeated column chromatography and preparative TLC using silica gel on $CHCl_3$ soluble fractions resulted in the isolation and characterization of five compounds including *ciryneol C* **1**, *scopoletin* **2**, *pectolinarigenin-7-0-β-glucopyrannoside* **3**, *acacetin* **4** and *6,7-Dimethoxycoumarin* **5**.

These compounds were screened for anti-bacterial activity against *B. subtillis, E. coli, S. flexenari, S. aureus, S. typhi, and P. aeruginosa.* The inhibition zones of **1**, **3** and **4** were almost the same and showed high activity in killing the *Bacillus subtilis* and *Shigella flexenari*. In *Staphylococcus aureus* and *Salmonella typhi,* the area of inhibition zone was same and showed high activity in **4** and low activity in **1**, **2**, **3** and **5**. In case of *Escherichia coli* and *Pseudomonas aeruginosa, the inhibition zones were the same showing low activity in **1, 2, 3, 4** while **5** remained totally ineffective due to the resistance* (Table 1).

Table 1. Antibacterial activity of compounds **1-5** from *Cirsium arvense.*

Bacteria	**1**	**2**	**3**	**4**	**5**	Imepinem
Bacillus subtillis	28	20	30	35	11	35
Escherichia coli	20	10	11	18	-	32
Shigella flexenari	32	18	28	32	10	35
Staphylococcus aureus	20	12	10	33	12	34
Salmonella typhi	22	11	10	33	10	35
Pseudomonas aeruginosa	16	10	10	18	-	30

Note: Temperature 37 ^{o}C, Values are inhibition zones (mm) and an average of triplicate. Concentrations used were in 1000 µg /mL.

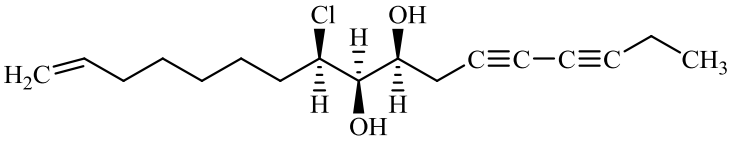
The fungicidal activity of **1-5** was performed against Six pathogenic fungi, *Trichophyton longifusus, Candida albicans, Aspergillus flavus , Microsporum canis, Candida glaberata and Fusarium solani.* The results (Table 2) indicated that these compounds **1-5** were not highly active against these fungi, except **2** and **3,** which exhibited moderate activity while **1** and **4** showed low activity in killing the *Trichophyton longifusus, Candida albicans, Microsporum canis and Fusarium solani.*

It was further observed that **1-4** were weak active against *Aspergillus flavus* and *Candida glaberata* and **5** was devoid of any antifungal activity against the rest of the tested fungi.

Table 2. Antifungal activity of compounds **1-5** from *Cirsium arvense.*

Microorganism	Zone of Inhibition Diameter (mm)					Standard drug
Fungi	**1**	**2**	**3**	**4**	**5**	Amphotericin B20
Trichophyton longifusus	10	10	25	12	-	40
Candida albicans	10	25	11	10	-	30
Aspergillus flavus	10	11	12	10	-	40
Microsporum canis	8	10	8	8	-	20
Candida glaberata	14	10	14	12	-	50
Fusarium solani	10	23	12	10	-	45

Note: Temperature 37 ^{o}C, Values are inhibition zones (mm) and an average of triplicate. Concentrations used were 1000 µg /mL.



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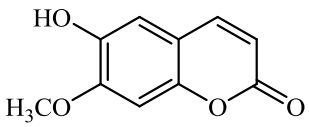

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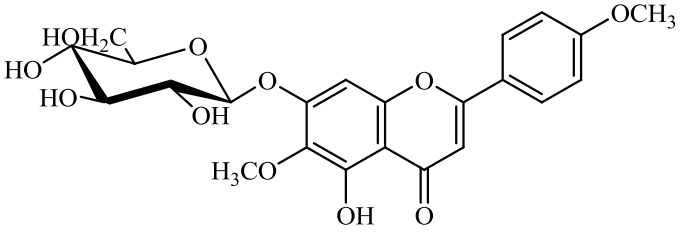

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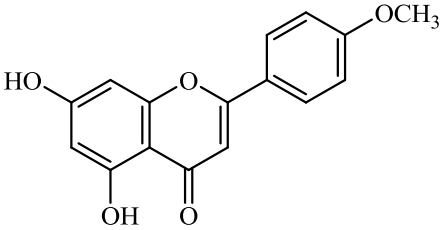

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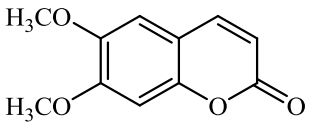

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Conclusion

Viral, bacterial and fungicidal diseases are great threat to humanity. Many current drugs are derived from isolated compounds from the plants and are now being routinely used in modern medicine. Natural compounds are preferable due to their non toxicity and easy biodegradation nature as compare to synthetic compounds. The diverse medicinal uses of *Cirsium arvense* and the results of the present investigation of the methanolic extract, two fractions ($CHCl_3$, EtOAc) and compounds **1**, **2**, **3**, **4** exerts significant toxicity, antibacterial and antifungal activities. These evidences direct us for further study to isolate the active compounds, to derive maximum therapeutic potential of the plant.

Experimental Section

General Experimental Procedure

TLC was performed on precoated silica gel F_{254} plates; Silica gel (E-Merck, 230-400 mesh) was used for Column chromatography. Melting points were determined on a Gallenkemp apparatus and are uncorrected. The UV spectra (λ_{max} nm) were recorded on Hitachi UV- 3200 spectrophotometer in MeOH. The IR spectra (ν_{max} cm^{-1}) were recorded on Jasco-320-A spectrophotometer in $CHCl_3$. The mass spectra were recorded on a Varian MAT 312 double focusing mass spectrometer connected to DEC–PDP 11 / 34 computer system. The ^1H and ^{13}C NMR spectra were recorded on a Bruker AM – 300 NMR spectrometer (300 MHz for ^1H and 75 MHz for ^{13}C NMR), using $CDCl_3$ as solvent. The assignments were made by DEPT, COSY and HMQC experiments. Optical rotations were measured on Jasco-DIP-360 digital polarimeter using a 10 cm tube. Ceric sulphate and aniline phthalate were used as detecting reagents.

Plant Material

The plant material was collected from Musa Khel Bannu and identified by Muhammad Yousf Khan, Professor in Botany, Govt Post Graduate Collage Bannu. The specimen (NO: 230) was deposited in the Herbarium of Botany Department in Govt Post Graduate Collage Bannu.

Extraction and Isolation

The shade dried plant of *Cirsium arvense* (8 kg) was ground and extracted with MeOH (32 X 3) at room temperature for seven days. The combined methanolic extract was evaporated under reduced pressure to yield a dark brown gummy material (650 g). The gummy material was suspended in water and extracted with *n*-hexane (115 g), $CHCl_3$ (98 g), EtOAc (82 g) and *n*-butanol (60 g) soluble fractions respectively. These fractions were screened for toxicity. The major toxicity was observed in $CHCl_3$ soluble fraction.

The Chloroform soluble fraction was subjected to column chromatography over silica gel (70-230 mesh) eluting with *n*-hexane, *n*-hexane-$CHCl_3$, $CHCl_3$, $CHCl_3$-EtOAc, EtOAc, EtOAc-MeOH and MeOH in increasing order of polarity to obtain sub-fractions A-F.

The sub-fraction B (100 % $CHCl_3$, 11 g) was again chromatographed over silica gel eluting with *n*-hexane, $CHCl_3$, $CHCl_3$ and $CHCl_3$- EtOAc in increasing order of polarity to obtain four fractions A´- D´. The fraction C´ (2.4 g) obtained from $CHCl_3$: EtOAc (1:1) was again subjected to column chromatography over silica Gel eluting with mixture of *n*-hexane: $CHCl_3$ and $CHCl_3$: EtOAc in increasing order of polarity. The fractions which eluted with

$CHCl_3$: EtOAc (9:1) were combined and loaded on preparative TLC in solvent system *n*-hexane : Acetone : Ethanol (5:4:1) yielded *ciryneol* (**1**, 19 mg).

The fractions D´ obtained from EtOAc (100 %) was again subjected to column chromatography over silica gel eluting with mixtures of n-hexane: $CHCl_3$ and $CHCl_3$: EtOAc in increasing order of polarity. The fractions obtained from $CHCl_3$: EtOAc (5.5:4.5) showed a major spot on TLC and subsequent preparative TLC using *n*-hexane: Ethanol: Diethylamine (8:2:3 drops) as solvent system Afforded *scopoletin* (**2**, 18 mg).

The sub-fraction C (3.2 g) obtained from $CHCl_3$: EtOAc (1:1) was re-chromatographed over silica gel eluting with mixture of *n*-hexane: $CHCl_3$ and $CHCl_3$: EtOAc in increasing order of polarity. The fractions obtained from $CHCl_3$ (100 %) were mixed and showed a major spot on TLC. It was concentrated and subjected to preparative TLC using *n*-hexane: EtOAc (4.5:5.5) as solvent system to afford *pectolinergenin-7-O-β-glucopyranoside* (**3**, 21 mg).

The sub-fraction D (100 % EtOAc, 10.7 g) was re-chromatographed over silica gel eluting with $CHCl_3$: EtOAc and EtOAc: EtOH in increasing order of polarity. The fractions obtained from $CHCl_3$: EOAc (2.5:7.5) were mixed and concentrated. The concentrated material (2.4 g) was again subjected to column chromatography over silica gel eluting with mixture of $CHCl_3$: EtOAc in increasing order of polarity. The fractions obtained from $CHCl_3$: EtOAc (3.5:6.5) were subjected to preparative TLC using *n*-hexane: Acetone: EtOAc (2:2:6) as solvent system to furnish two compounds, *Acacetin* (**4**, 20 mg) and *6,7-Dimethoxycoumarin* (**5** , 16 mg) from the top and tail of the fraction respectively.

***Ciryneol C* (1);** Colourless Oil**,** BP: 66-70 °C; Yield: 0.02 %.
The spectral data showed complete agreement with those reported in literature[8].

***Scopoletin* (2);** Light yellow needles; mp: 140-144 °C; Yield: 0.2 %.
The spectral data showed complete agreement with those reported in literature[8].

Pectolinergenin 7-O-β-glucopyarnoside* (3);* White amorphous powder; mp: 156-158 °C; Yield: 0.21 %. The spectral data showed complete agreement with those reported in literature[10].

***Acacetin* (4)**; Yellow needles, mp: 260-266 °C; Yield: 0.2 %.
The spectral data showed complete agreement with those reported in literature[10].

***6, 7-Dimethaxycoumarin* (5);** Light yellow needles, mp: 143-145 °C; Yield: 0.16 %,
The spectral data showed complete agreement with those reported in literature[22].

Antimicrobial Activities:

Antibacterial assay

The antibacterial activities were determined using ager well diffusion method [23]. Bacterial culture was grown in nutrient broth at 37°C for 18-24 hours. 0.5 mL of broth culture of test organism was added by sterile pipette in to molten agar (50 mL) which were than cooled to 40 °C and poured in to sterile Petri dish. Sterile cork borer were used to make well of 6 mm in diameter in nutrient ager plate. The wells were filled with given compounds of (100 µL) and the plate was allowed for 1-2 hours. The plates were incubated at 37 °C for 18-24 hours. Finally the diameter of inhibition was measured.

Anti-fungal assay

The antifungal assay was carried out using ager well diffusion method[23]. Sterile DMSO was used to dissolve the test sample. SDA (Sabouraud dextrose agar) was prepared by mixing Sabouraud 3 % glucose agar and agar-agar in distilled water. The required amount of fungal strain was suspended in 2 mL Sabouraud dextrose broth. This suspension was uniformly streaked on Petri plates containing Sabouraud dextrose agar media by means of sterile cotton swab. Compounds were applied in to well using same technique for bacteria. These plates were than seen for the presence of zone of inhibitor and result was noted.

References

1 - H. P. Alley, N. E. Humburg. Res. J. Wyoming Agric. Exp. Sta., **1977**, *112*, 44–47.

2 - S. Bent, A. Padula, D. Moore, M. Patterson, Transline Herbicide Product Label, **2005**, *8*, 462-468.

3 - C. W. Grekul and E. W. Bork, Weed Tech., **2004**, *18*, 784-794.

4 - L. B. Nadeau, W. H. Vandenborn, Weed Sci., **1990**, *38*, 379-384.

5 - C. W. Grekul, E. W. Bork, Crop Prot., **2007**, *26*, 668-676.

6 - R. Eschen, S. Hunt, C. Mykura, A.C. Gange and B.C. Sutton, Fungal Biology, **2010**, *114*, 991-998.

7 - S. Dang, *Plants Profile for Cirsium vulgare*, USDA Plant Database: Seoul, **1984**.

8 - S. H. Yim, H. J. Kim, I. S. Lee. Arch. Pharm. Res., **2003**, *26* (2), 128-131.

9 - D. Moerman, L. S. Marks, A.W. Partin, J. I. Epstein, V. E. Tyler, I. Simon, M. L. Macairan, Native American Ethnobotany: Timber Press, Oregon**, 1998**.

10 - Chun-nan Lin et al., Chem. Pharm. Bull., **1978**, *26* (7), 2036-2039.

11 - V. Nuzzo. Element Stewardship Abstract for Cirsium arvense. The Nature Conservancy Arlington, VA. **1997**.

12 - L. Cavalca, R. Zanchi, A. Corsini, M. Colombo, C. Romagnoli, E. Canzi, V. Andreoni, Systematic and Applied Microbiology, **2010**, *33*, 154-164.

13 - A. Berestetskiy, A. Dmitriev, G. Mitina, I. Lisker, A. Andolfi, A. Evidente, Phytochemistry, **2008**, *69*, 953-960.

14 - Nina Theis, J. Chem. Ecol., **2006**, *32*, 917-927.

15 - M. Ganzera, A. Pocher and H. Stuppner, Phytochem. Anal., **2005**, *16*, 205-209.

16 - J. Nazaruk, P. Jakoniuk. J. Ethnopharmacol., **2005**, *102*, 208.

17 - A. B. Bohm, T. F. Stuessy. Flavonoids of the Sunflower Family (Asteraceae). Wien: Springer-Verlag, **2001**.

18 - I. E. Jordon Thaden, S. M. Louda, Biochem. Syst. Ecol., **2003,** *31*, 1353.

19 - J. Nazaruk, J. Gudej, Acta Polon Pharm Drug Res., **2003**, *60*, 87.

20 - J. Nazaruk, Fitoterapia, **2008**, *79*, 194-196.

21 - B. N. Meyer, N. R. Ferrigni, J. E. Putnam, L. B. Jacobsen, D. E. Nichols, and J. I. Mclaughlin, Planta Medica, **1982**, *45*, 31.

22 - N. Mortia and C.N. Lin. J. Taiwn. Pharm. Assoc., **1976**, *28*, 40.

23 - Y. K. Boakye, N. Fiagbe, J. Ayim. J. Nat. Prod., **1977**, *40*, 543-545.