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In vitro antimicrobial activity of the chemical constituents of *Cirsium arvense* (L). Scop

Zia Ul Haq Khan[1], Shafiullah Khan[2], Yongmei Chen[1] and Pingyu Wan[1]*

[1]School of Science, Beijing University of Chemical Technology, Beijing, 100029, China.
[2]School of Material Science and Engineering, Beijing University of Chemical Technology, Beijing 100029, China.

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Anti-bacterial and anti-fungal activities of the isolated constituents from *Cirsium arvense* were evaluated. These five compounds namely α-tocopherol, 9, 12, 15-octadecatrienoic acid, tracin, hispidulin and luteolin were isolated from the ethyl acetate soluble fraction of the methanolic extract of the plant. Their structures have been identified by electrochemical impedance spectroscopy (EIS), high resolution resonance ionization mass spectrometry (HR-EIMS), ^{1}H-NMR and ^{13}C-NMR spectroscopic methods. The antibacterial and antifungal activities of these compounds were tested; compounds 3, 4 and 5 shows good activities.

Key words: *Cirsium arvense*, fractionation, isolation, antibacterial activity, antifungal activity.

INTRODUCTION

Cirsium arvense is a medicinal plant of family Asteraceae (Alley et al., 1977) and is often found as noxious weed in grasslands and riparian habitats (Bent et al., 2005). *C. arvense* is known to reduce forage biomass (Grekul et al., 2004) as well as its favorable response to fertilization (Nadeau et al., 1990; Grekul et al., 2007). A recent study shows that the foliar endophytic fungal community composition in *C. arvense* is affected by mycorrhizal immigration and soil nutrient content (Eschen et al., 2010). The genus *Cirsium* L. have various medicinal uses especially for the treatment of peptic ulcer and leukaemia in folk medicine (Dang et al., 1984), epitasis, metrorrhagia, syphilis eye infections (Lee et al., 2002), skin sores gonorrhoea, bleeding piles and has also been found to be effective against diabetes (Jolanta et al., 2003; Won et al., 1978). Americans and Indians allegedly used an infusion of *C. arvense* roots for mouth diseases, worms and poison-ivy (*Toxicodendron radicans*) and in treatment of tuberculosis (Nuzzo et al, 1997). *C. arvense* roots are reported to having arsenic-resistant bacteria (Cavalca et al., 2010). A recent study discovered that nonenolides and cytochalasins exhibited strong phytotoxic activity against *C. arvense* leaves (Berestetskiy et al., 2008). Scent of *C. arvense* attracts both floral herbivores and pollinators (Andrews et al., 2007).

C. arvense is found in very considerable quantities in district Bannu Pakistan. Its local name is "*Aghzikai*". Prior studies recommended that flavonoid compounds, phenolic acids, tannins, sterols and triterpenes are the main constituents of genus *Cirsium* (Nazaruk et al., 2005; Bohm et al., 2001; Jordon et al., 2003). The various therapeutic uses attributed to this species prompt us to carry out phytochemical exploration and biological activities of constituents of this plant. The methanolic extract of the whole plant of *C. arvense* showed significant toxicity in brine shrimp lethality test (Meyer et al., 1982).

*Corresponding author. E-mail: pywan@mail.buct.edu.cn or ziaulhaqkhan11@gmail.com. Fax: 010- 64435452.

In our previous work, several antibacterial and antifungal compounds have been isolated from the chloroform fraction of the methanolic extract of the plant (Zia Ul Haq khan et al., 2011). Here, we report our further work on EtOAc fraction. After being separated by column chromatographic method, five compounds were isolated and their antibacterial and antifungal activities were tested. These compounds are first time isolated from the plant.

MATERIALS AND METHODS

General experimental procedure

The precoated silica gel F_{254} plates were used for TLC, Silica gel (E-Merck, 230-400 mesh) was used for column chromatography. Melting points were determined on a Gallenkemp apparatus and are corrected. 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-320A 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 systems. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker AM-300 NMR spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C-NMR) using $CDCl_3$ as solvent. The assignments were made by distortionless enhancement by polarization transfer (DEPT), correlation spectroscopy (COSY) and heteronuclear multiple-quantum correlation (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

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

Extraction and isolation

The shade dried plant of *C. arvense* (8 kg) was ground and extracted with MeOH (32 L×3) at room temperature. The combined methanolic extract was evaporated under reduced pressure to obtain 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. The fractions were then placed in a vacuum oven at not more than 40°C for about 24 h to remove any residual solvent. These fractions were screened for toxicity. The $CHCl_3$ and EtOAc soluble fractions show highly toxicity (Zia Ul Haq khan et al., 2011). The ethyl acetate (EtOAc) soluble fraction was subjected to column chromatography over silica gel (70-230 mesh) eluting with *n*-hexane, *n*-hexane:EtOAc, EtOAc:$CHCl_3$ and EtOAc, EtOAc:MeOH and MeOH in increasing order of polarity to obtained sub-fractions (A to F).

The sub-fraction B (100% EtOAc,15 g) was again chromatographed over silica gel eluting with *n*-hexane-EtOAc, EtOAc-$CHCl_3$ and EtOAc in increasing order of polarity to obtain four fractions A' to D'.

The fraction C' (2.4 g) obtained from EtOA:$CHCl_3$ (6:4) was again subjected to column chromatography over silica gel eluting with mixture of *n*-hexane: EtOAc-$CHCl_3$ and EtOAc, in increasing order of polarity. The fractions eluted with EtOAc:$CHCl_3$ (7:3) were combined and loaded on preparative thin layer chromatography (TLC) in solvent system *n*-hexane: Acetone: Ethanol (4:4:2) yielded, α-tocopherol (compound 1, 10 mg). The fraction D' obtained from EtOAc (100%) was again subjected to column chromatography over silica gel eluting with mixtures of *n*-hexane: EtOAc:$CHCl_3$ and EtOAc in increasing order of polarity.

The fractions obtained from EtOAc:$CHCl_3$ (6.4:3.7) showed a major spot on TLC and subsequent preparative TLC using *n*-hexane:ethanol:diethyl amine (6:4:3) as solvent system, afforded 9, 12, 15-octadecatrienoic acid (compound 2, 17 mg). The sub-fraction C (3.2 g) obtained from EtOAc:$CHCl_3$ (6:4) was re-chromatographed over silica gel eluting with mixture of *n*-hexane: EtOAc: $CHCl_3$ and EtOAc in increasing order of polarity. The fractions obtained from EtOAc (100%) were mixed and showed a major spot on TLC. It was concentrated and subjected to preparative TLC using *n*-hexane:EtOAc (3.7:6.3) as solvent system to afford tracin (compound 3, 18 mg).

The sub-fraction D (100% EtOAc 11 g) was re-chromatographed over silica gel eluting with *n*-hexane:EtOAc, EtOAc:$CHCl_3$, EtOAc and EtOH in increasing order of polarity. The fractions obtained from EtOAc:$CHCl_3$ (2.7:7.3) were mixed and concentrated. The concentrated material (1.8 g) was again subjected to column chromatography over silica gel eluting with mixture of EtOAc:$CHCl_3$ in increasing order of polarity. The fractions obtained from EtOAc:$CHCl_3$ (2.7:6.3) were subjected to preparative TLC using *n*-hexane:acetone:EtOAc (1.5:2.5:6) as solvent system to furnish two compounds, hispidulin (compound 4, 12 mg) and luteolin (compound 5, 11 mg).

α-Tocopherol (compound 1)

Pale yellowish oil BP: 106 to 110°C. The spectral data showed complete agreement with those reported in literature (Lee et al., 2002).

9, 12, 15-Octadecatrienoic acid (compound 2)

Colorless gum; MP: 69 to 78°C. The spectral data showed complete agreement with those reported in literature (Lee et al., 2002).

Tracin (compound 3)

Yellow needle; MP: 256 to 265°C. The spectral data showed complete agreement with those reported in literature (Jolanta et al., 2003).

Hispidulin (compound 4)

Yellow needles; MP: 260 to 266°C. The spectral data showed complete agreement with those reported in literature (Lee et al., 2002).

Luteolin (compound 5)

Light yellow needles; MP: 258 to 270°C. The spectral data showed complete agreement with those reported in literature (Jolanta et al., 2003).

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

Types of bacteria	Zone of inhibition diameter (mm)					
	1	2	3	4	5	Imepinem
Bacillus subtillis	18	11	34	31	33	35
Escherichia coli	15	08	27	29	31	32
Shigella flexenari	17	11	29	31	33	35
Staphylococcus aureus	14	09	26	29	32	34
Salmonella typhi	17	11	28	30	32	35
Pseudomonas aeruginosa	16	13	28	29	29	30

Temperature, 37°C. Values are inhibition zones (mm) and an average of triplicate. Concentrations used were in 1000 µg/ml.

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

Microorganism	Zone of inhibition diameter (mm)					
	1	2	3	4	5	Amphotericin B20
Trichophyton longifusus	16	04	26	31	34	40
Candida albicans	13	--	19	24	27	30
Aspergillus flavus	21	02	19	29	35	40
Microsporum canis	13	--	--	--	15	20
Candida glaberata	25	05	22	32	37	50
Fusarium solani	19	05	22	36	38	45

Temperature, 37°C. Values are inhibition zones (mm) and an average of triplicate. Concentrations used were 1000 µg/ml.

Antimicrobial activity

Antibacterial assay

The antibacterial activities were determined using agar well diffusion method (Boakye et al., 1977). Bacterial culture was grown in nutrient broth at 37°C for 18 to 24 h. 0.5 ml of broth culture of test organism was added by sterile pipette into molten agar (50 ml) which were then cooled to 40°C and poured into sterile petri dish. Sterile cork borer were used to make well of 6 mm in diameter in nutrient agar plate. The wells were filled with given compounds of (100 µl) and the plate was allowed for 1 to 2 h. The plates were incubated at 37°C for 18 to 24 h. Finally, the diameter of inhibition was measured.

Antifungal assay

The antifungal assay was carried out using agar well diffusion method (Hadacek et al., 2000). Sterile dimethyl sulfoxide (DMSO) was used to dissolve the test sample. Sabouraud dextrose agar (SDA) 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 SDA media by means of sterile cotton swab. Compounds were applied into well using same technique for bacteria. These plates were then seen for the presence of zone of inhibitor and result was noted.

RESULTS AND DISCUSSION

The *n*-hexane, $CHCl_3$, EtOAc and *n*-BuOH soluble fractions were taken of methanolic extract. The repeated column chromatography and preparative TLC using silica gel on ethyl acetate soluble fractions resulted in the isolation and characterization of five compounds, namely (compound 1) α-tocopherol, (compound 2) 9,12,15-octadecatrienoic acid, (compound 3) tracin, (compound 4) hispidulin and (compound 5) luteolin, respectively.

The antibacterial activity of these compounds was performed against *Bacillus subtillis, Escherichia coli, Shigella flexenari, Staphylococcus aureus, Salmonella typhi,* and *Pseudomonas aeruginosa.* As shown in Table 1. The zone of inhibition of compounds 3, 4 and 5 were almost the same as those of imepinem and much larger than those of compounds 1 and 2, which means compounds 3, 4 and 5 have high activity in killing *B. subtilis*, *E. coli, S. flexenari, S. aureus,* and *S. typh*i, and compound 1 shows moderate activity and compound 2 show low activity.

The antifungal activity of compounds (1 to 5) was performed against six pathogenic fungi, *Trichophyton longifusus, Candida albicans, Aspergillus flavus, Microsporum canis, Candida glabrata* and *Fusarium solani*

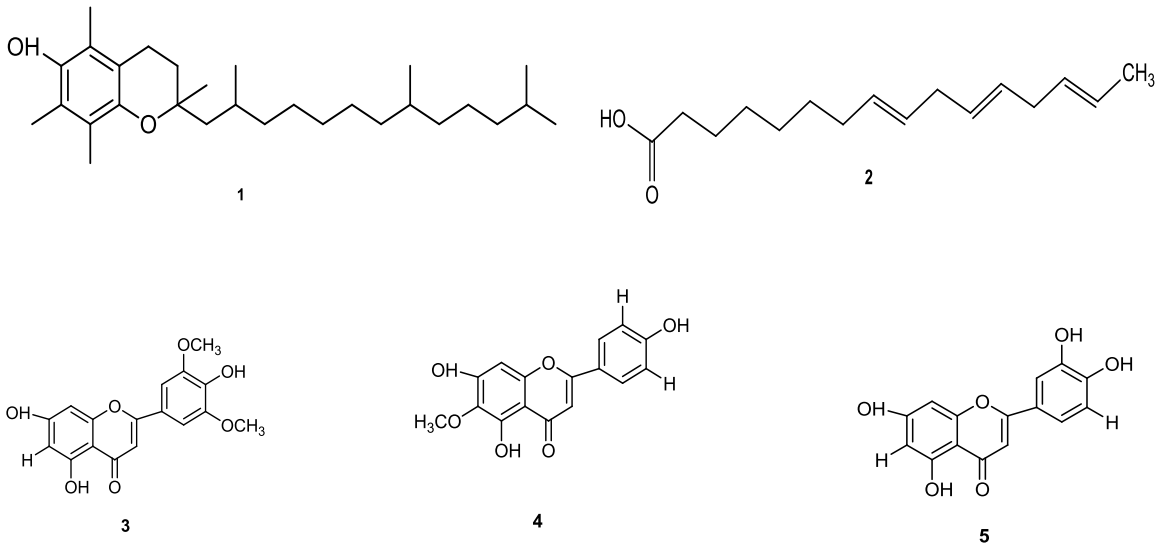

Figure 1. Structures of compounds 1 to 5.

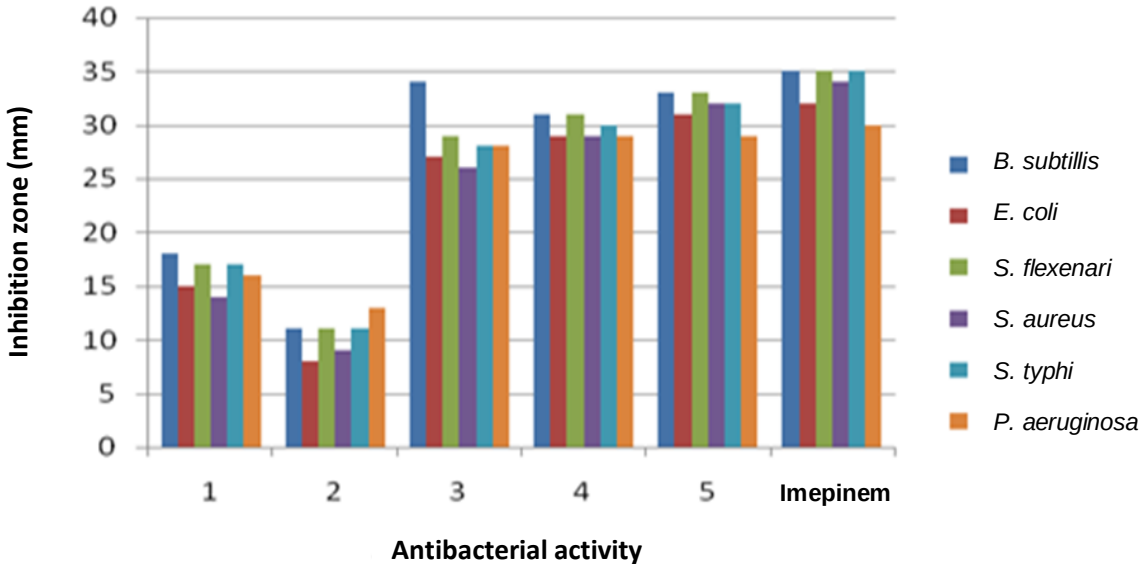

Figure 2. Antibacterial activity of compounds 1 to 5 isolated from *Cirsium arvense.*

(Table 2). With the contrast experiment of Amphotericin B20, compounds 1 to 5 showed less antifungal activity than that of Amphotericin B20. However, compounds 3, 4 and 5 showed satisfied antifungal activity to all of six fungi and compound 1 have moderate activity while compound 2 has no antifungal activity. Figures 1, 2 and 3.

Conclusion

Tracin, hispidulin and luteolin were isolated from *C. arvense*, which showed high antibacterial activity against *Staphylococcus aureus* and *Salmonella typhi bacteria*. They may be used against different human ailments. It was concluded that the *C. arvense* have potential antimicrobial activity. This plant is used as one of the ingredients of traditional medicine in some parts of the world. Further investigations are recommended to exploit the hidden medicinal value of this plant.

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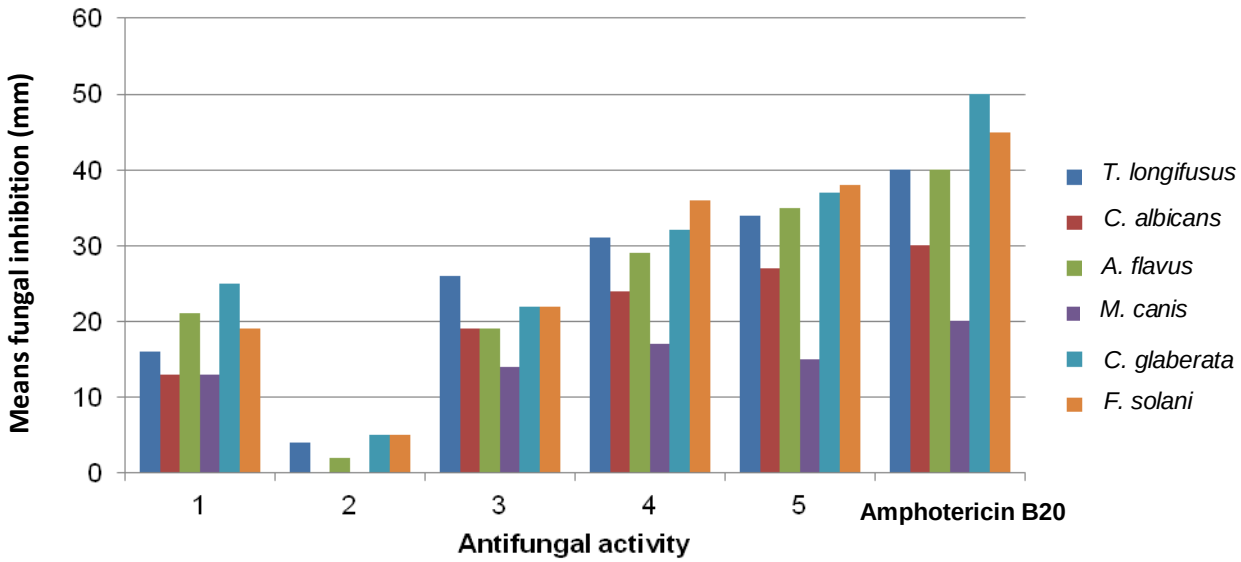

Figure 3. Antifungal activity of compounds 1 to 5 isolated from *Cirsium arvense.*

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