Iranian Journal of Pharmaceutical Sciences
2016: 12 (2): 1-8
www.ijps.ir

Original Article

TLC Screening and Evaluation of Antioxidant, Antibacterial Activity of *Onopordon Macrocephalum* by Bioautography Method

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Abstract

Onopordon known as scotch thistle is a medicinal plant of the Asteraceae family that is widely distributed in Europe and Asia, also it has many valuable medicinal properties as hypotensive and antitumor. Thin layer chromatography (TLC) is wildly used in natural product extract analysis as a finger print which can be used for identification and quality control of medicinal preparations. Performing Thin-layer chromatography TLC tests on the plant shows the presence of flavonoids, coumarins, and bitter principles. Planar chromatographic analysis hyphenated with the biological detection method is known as bioautography which is an effective and inexpensive technique for the phytochemical analysis of plant extracts. Thin-layer chromatography-direct bioautography links separation on the adsorbent layer with biological tests performed directly on it in order to identify antimicrobial and antioxidant activities. Bioautography revealed that *Staphylococcus aureus* was inhibited by most of the flavonoids separated on the TLC plates. Similarly, growth of the *Bacillus cereus* was also inhibited by one flavonoid band on TLC. Antioxidant bioautography shows the strong antioxidant activity of one flavonoid band, whereas no activity detected of bitter principles. Bioautography showed that the antimicrobial and antioxidant activity was probably due to flavonoids.

Keywords: antioxidant, bioautography, flavonoids, Onopordon macrocephalum, TLC.

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Cite this article as: Sharif S, Kitaz A, Al-Kayalib R, TLC Screening and Evaluation of Antioxidant, Antibacterial Activity of Onopordon Macrocephalum by Bioautography Method.Iranian Journal of Pharmaceutical Sciences, 2016, 12 (2): 1-8.

1. Introduction

Plant-derived substances have recently become of great interest owing to their versatile applications. It has been estimated that 14 - 28% of higher plant species are used medicinally and that 74% of pharmacologically active plant derived components were discovered after following up on ethnomedicinal use of the plants [1]. Medicinal plants contain a vast combination of phytochemicals such as phenolic acids, flavonoids, tannins, lignans, and other compounds that show several therapeutic effects [2]. The genus

Onopordon is one of the most important Plants belonging to Asteraceae family and it has been employed traditionally for its antibacterial, hemostatic, and hypotensive properties and for the treatment of skin cancer. *Onopordon* genus is known to produce a variety of active compounds, including flavonoids, sesquiterpenes, flavonolignans and phenylpropanoids [2, 3]. Various studies demonstrated the antioxidant properties of other *Onopordon* specious as *Onopordon acanthium* in Hungary [4] and *Onopordum alexandrinum* in Egypt showed significant hepatoprotective and free radical scavenging effects [5]. In addition, antibacterial activity of some *Onopordon* specious was assessed, and they have a good activity on gram positive bacteria strains as *S. maltophilia* and *S. aureus* [3, 6].

The isolation and identification of secondary metabolites produced by plants are important steps on the way to use these compounds as active principles in medicinal preparations. One of the most effective and inexpensive techniques of plant extracts analysis is bioautography. Bioautography in other words is the hybridization between planar chromatographic analysis with the biological detection method [7]. It can thus be performed both in highly developed laboratories as well as in small research laboratories. Bioautography offers a simple, rapid and inexpensive method for the chemical and biological screening of complex plant extracts [7].

In this study we aimed to identify the various phytoconstituents and detect the components in the *Onopordon* crude extract that are responsible for antioxidant and antibacterial activities.

2. Materials and Methods

1.1. Plant Samples

The aerial parts of *Onopordon macrocephalum* were collected during their flowering time in May 2014 from Al-muhandiseen in Aleppo. The plant sample was identified by Dr. Ahmad Jaddoh in the faculty of Agricultural Engineering, Aleppo University, Aleppo, Syria. Flowers were separated as floral part, while leaves and stems formed the vegetative part, then parts dried at room temperature with good ventilation under shade. The shade-dried parts were powdered using mechanical grinder, then the powder was used for extraction.

1.2. *Extraction Procedures*

The powdered plant sample was extracted by Ultrasonication Assisted Extraction with five folds of methanol/water (80:20, *v/v*) at 40^{o}C. The extract was filtered through Whatman NO.1 papers. The residue was then re-extracted twice with the same volume of methanol/water (80:20, *v/v*). The combined extracts were evaporated at 40°C (rotary evaporator Büchi R-215, Flawil, Switzerland) to remove methanol. The obtained extract was evaporated under reduced pressure and kept in a vacuum desiccator to remove traces of solvents. The crude extract was kept separately in sterile sample tubes and stored at 4 °C [8, 9] .

1.3. Bacterial Strains

Bacterial strains include *Staphylococcus aureus* ATCC25923 and *Bacillus cereus* ATCC11778.

2.4. Thin Layer Chromatography TLC

TLC is wildly used in natural product extract analysis as a finger print which can be used for

identification and quality control of medicinal preparations. Three types of extracts were prepared by 1g of powdered plant with three solvents: 5ml of methanol, 10ml dichloromethane and 5ml of methanol after dealing with 1ml of 10% ammonia. Chemical constituents of the extracts were separated on aluminum-backed thin layer chromatography (TLC) plates (Merck, silica gel 60 F254).

The TLC plates were developed under saturated conditions with one of the two eluent systems developed in our laboratory, i.e., ethyl acetate/methanol/water (100:13.5:10) (polar); toluene/ethyl acetate (93:7) (non-polar). Separated chemical compounds were examined firstly using UV- 254nm and 365nm to detect florescent zones. After that, Separated chemical compounds sprayed with spray reagents in order to reveal spots of different groups using 10% ethanolic KOH for coumarins, natural product- poly ethylene glycol (NP-PEG5%) reagent for flavonoids, Dragendorff reagent for alkaloids and Vanillin-sulphuric acid reagent for terpenes and bitter principles[10, 11, 12].

2.5. Detection of antibacterial agents by bioautography

Bioautography was performed with a culture of *Staphylococcus aureus* ATCC25923 and *Bacillus cereus* ATCC11778 which showed a good sensitivity to the methaolic extract of vegetative part. Developed TLC plates of methanolic extract were prepared in ethyl acetate/methanol/water/glacial acetic acid/ formic acid (63:4:5:2:1) as mobile phase to separate four bands of flavonoids. Furthermore, other TLC plates were prepared in toluene/ethyl acetate (93:7) to separate four bands of bitter principles. Developed TLC plates were carefully dried for complete removal of the solvents and sprayed with or dipped into a fungal or bacterial suspension. Okusa *et al*, incorporated a new medium for direct TLC bioautography which is fluid enough to disperse microorganisms and viscous enough to adhere to the TLC plates; according to them a mixture of Muller–Hinton (MH) broth and MH agar in the ratio of 90:10 fulfills this requirement [7]. The plates were incubated for 24h at 37 C under humid condition and then sprayed with an 2mg/ml aqueous solution of 2, 3, 5-triphenyltetrazolium chloride. Then zones of inhibition, colored yellow, were compared with the R_F of the related spots on the reference TLC plate [11, 13].

2.6. Detection of Antioxidant Agents by Bioautography

The stable 2,2-diphenyl-1-picrylhydrazylradical (DPPH) has an absorption maximum at 517nm, which decreases upon reduction through reaction with a radical scavenger. The corresponding color change can thus be observed in a TLC bioassay. The developed chromatogram is sprayed with a solution of 0.2% DPPH in methanol/ethanol. The plate is examined in daylight after 30 min. Free-radical scavengers appear as cream/yellow spots against a purple back ground [7, 9].

3. Results and Discussion

3.1. Thin Layer Chromatography TLC

Qualitative phytochemical screening is an essential step towards the discovery of new drugs as it provides the information regarding the presence of a particular secondary metabolite in the plant extract of clinical significance. The

presence of any significant bioactive natural product indicates the necessity of separation of the compounds through suitable chromatographic techniques. In the present study, both of two extracts i.e. methanolic and dichloromethane were checked by thin layer chromatography**.** After dealing with spray reagents, the yellow florescent after KOH 10% reagent indicates the presence of coumarins, whereas the greenish and orange florescent after NP-PEG indicates the presence of flavonoids. Furthermore, the detected violet bluish spots after dealing with vanillin-sulphuric acid and heating indicates bitter principles presence.

Hence, it has been proven that the two parts of *Onopordon macrocephalum* (floral part, vegetative part) contain a diverse classes of bioactive compounds such as coumarins, flavonoids, flavonolignans and bitter principles as shown in figure 1. Besides of absence of alkaloids as in other species *O.acanthium* and *O.caricum*, these results agreed with the literature review on the plant which showed these chemical constituents to be present [14, 6]. These constituents responsible for a various pharmacological properties and many reports are available about the antiviral, antibacterial, antifungal, and anti-inflammatory properties [3].

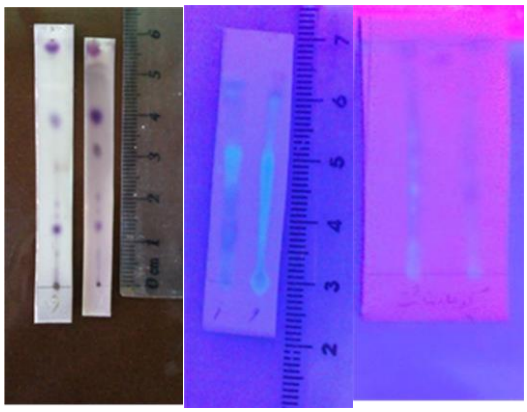

Figure1. Results of TLC screening plates of *O.macrocephalum* parts, vegetative part to the right and floral part to the left.

3.2. Detection of Antibacterial Agents by Bioautography

The chromatograms of methanolic extract of *O.macrocephalum* vegetative part were developed in order to calculate R_f values of the spots (Table1).

Table 1. Separated compounds of methanolic *extract of O.macrocephalum* vegetative part on TLC and their R_F values.

Plant extract	Detection reagent	R_f values
Methanolic extract	NP-PEG	0.461, 0.569, 0.692, 0.846
	Vanillin-sulphuric acid	0.161, 0.290, 0.419, 0.548

Developed TLC of separated flavonoids showed a good antibacterial activity. All separated flavonoids revealed yellow inhibition zones on *Staphylococcus aureus* ATCC25923 (Figure 2), whereas only one flavonoid band revealed an antibacterial activity on *Bacillus cereus* ATCC11778 (figue 3). Hence, the separated bitter principles did not show any inhibition zone on the two studied bacteria, but the inhibition was exclusive in the loading line which refers to the antibacterial effect of residual components apart from the separated bitter principles (Figure 2, Figure 3).

It was found that *Onopordon macrocephalum* had a total flavonoid content in methanolic extract of vegetative part reached to 93.54mg of RE/g

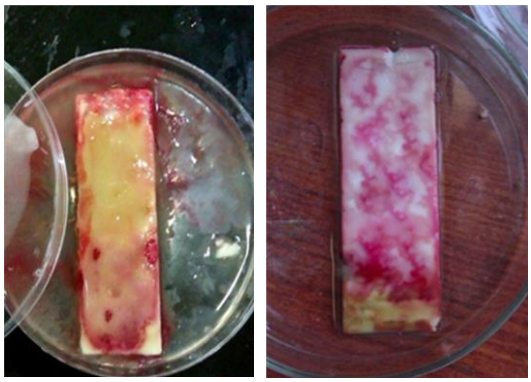

Figure 2. Antibacterial activity of methanolic extract of *O.macrocephalum* vegetative part by bioautography on *Staph.aureus*; A. flavonoids B. bitter principles.

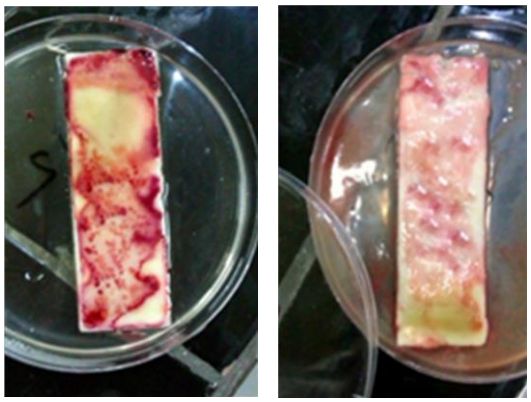

Figure 3. Antibacterial activity of methanolic extract of *O.macrocephalum* vegetative part by bioautography on *Bacillus cereus*; A. flavonoids B. bitter principles.

extract [15]. It should not be surprising that Flavonoids have been found *in vitro* to be effective against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular soluble proteins and to complex with bacterial cell walls. More lipophilic flavonoids may also disrupt microbial membranes [16].

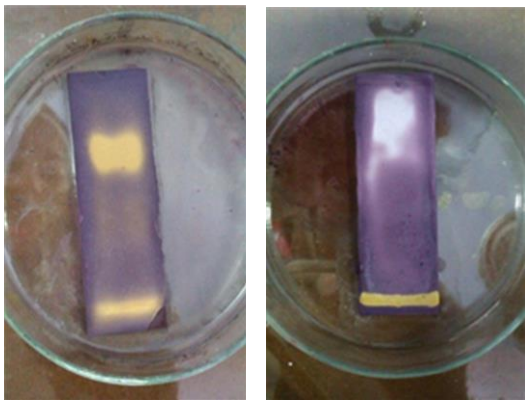

Figure 4. Antioxidant activity of methanolic extract of *O.macrocephalum* vegetative part by bioautography; A. flavonoids B. bitter principles.

3.3. Bioautography using DPPH as detection reagent

The reaction has been depicted in (Figure 4). It shows a strong antioxidant activity of one separated flavonoid (RF=0.69), whereas separated bitter principles did not show antioxidant activity and the effect was restricted in the loading line.

A study on isolated compounds of *Onopordon acanthium* showed that isolated flavonoids exerted pronounced effects, while lignans (bitter principles) were observed to be inactive. Furthermore, the study revealed that flavonoids have dual effect, acting firstly by inhibition of xanthine oxidase, resulting in decreasing generation of reactive oxygen species, and secondly by scavenging free radicals [4].

Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds such as flavonoids [17]. Since flavonoids are biological antioxidants, they are good for the management of cardiovascular diseases and oxidative stress which has been linked to cancer, aging, inflammation, and neurodegenerative diseases [18].

4. Conclusion

The results obtained in this study suggest that the identified phytochemical compounds may be the bioactive constituents and *Onopordon macrocephalum* is proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

The results indicate that the flavonoids in *Onopordon macrocephalum* methanolic extract of vegetative part had antibacterial and antioxidant properties. These findings support the use of this plant in folk medicine for the treatment of some diseases that are related to bacterial infections and liver diseases. Moreover, this study did the first steps for further studies that aimed to isolate and characterize active compound(s) responsible for the antibacterial and antioxidant activities from *Onopordon macrocephalum.*

Acknowledgements

Authors are thankful to University of Aleppo, Aleppo, Syria, for providing the infrastructure and necessary research facilities to carry out the research work.

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