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First Extraction of Onopordum Cyrenaicum Maire and Weiller and It's Antibacterial Activity

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الاستخلاص الأولي لنبات أونوبوردوم سيرينايكوم مير وويلر ونشاطه المضاد للبكتيريا

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Abstract:
Herbal medicines and their derivatives are best available from raw plant extracts. Onopordum cyrenaicum Maire & Weiller: medicinal plant endemic to Libya, Asteraceae species in Onopordum genus. The phytochemical profiling and antibacterial activity of this plant are investigated for the first time in this study. Slight modification of maceration technique was used to extract bioactive components step by step using 3 different solvents (petroleum ether, chloroform and ethanol). We have performed the phytochemical analysis for the detection of various components, which were terpenoids, tannins, glycosides, flavonoids, saponins and alkaloids. Of note, the ethanol extract possessed the highest antibacterial action when compared to other extracts, yielding inhibition zones of 28 mm (S. aureus), 22 mm (MRSA), and 30 mm (S. epidermidis); while being 6.25, 50, and 3.75 mg/ml as the minimum inhibitory concentrations (MIC) respectively.

Keywords: Onopordum cyrenaicum, phytochemicals, inhibition zone, antibacterial properties.

الملخص
تُعتبر المستخلصات النباتية الخام أفضل مصدر للأدوية العشبية ومنتجاتها المشتقة. تعد عشبة اندوروم سيرينايكم (الشوك البري)، وهو نوع ينتمي إلى جنس *Onopordum* من عائلة النجميات(Asteraceae) ، نباتًا طبيًا متوطنًا في ليبيا. تهدف هذه الدراسة إلى استكشاف المكونات الكيميائية النباتية والخصائص المضادة للبكتيريا لهذا النبات لأول مرة. تم استخدام تقنية النقع المعدلة قليلاً لاستخلاص المكونات النشطة بيولوجيًا باستخدام ثلاث مذيبات مختلفة (الإيثر البترولي، الكلوروفورم، والايثانول). كشف التحليل الكيميائي النباتي عن وجود عدة مركبات نباتية، بما في ذلك التربينات، العفص، الجليكوسيدات، الفلافونويدات، الصابونينات، والقلويدات. وأظهر مستخلص الايثانول نشاطًا مضادًا للبكتيريا بشكل استثنائي، متفوقًا على المستخلصات الأخرى، حيث بلغت مناطق التثبيط 28 ملم لـ *S. aureus*، و22 ملم لـ *MRSA*، و30 ملم لـ *S. epidermidis*، بينما كانت التركيزات المثبطة الدنيا 6.25 (MIC)، 50، و3.75 ملغ/مل على التوالي.

الكلمات المفتاحية :اندوروم سيرينايكم، المركبات الكيميائية النباتية، منطقة التثبيط، الخصائص المضادة للبكتيريا.

Introduction

Plants have formed an integral part of human life and survival for millennia, functioning as a primary source of food, medicine and other critical life supporting commodities [1]. Civilizations from ancient Egypt, China, and India used plants for a variety of ailments, a practice that remains today, represented by systems of traditional medicine, such as Ayurveda, Traditional Chinese Medicine, and Indigenous

medicine [2]. It should be noted that the transforming capabilities of plants are mainly attributable to their ranging phytochemicals [3], which are bioactive compounds found in plants. Phytochemicals, as a part of plant metabolism, are the secondary metabolites that plants produce which are proven to have several biological activities including antioxidant, anti-inflammatory, and anticancer activities [3, 4]. There are various plant families studied for their rich phytochemical content, but such studies are being most significant for the Asteraceae family due to its rich diversity and wide use in traditional medicine [5].

Onopordum is a genus of around 50 species of perennial seed-producing plants in the tribe Cardueae of the Asteraceae family [6]. Such morphological features as spiney leaves and high thistle-like structures guarantee that these plants are easily recognizable in their natural environments [7]. Historically, Onopordum species have been utilized to treat a range of human diseases, such as digestive disorders, skin diseases, and respiratory problems, attributed to their bioactive constituents [8]. One of the most distinguished species in this area is Onopordum cyrenaicum Maire & Weille, a Libyan endemic species characterized by both ecological and cultural importance [9]. However, no work has been conducted on Onopordum cyrenaicum, which created a gap in the research regarding the phytochemicals and the potential medicinal value [10].

Phytochemicals are a significant contributor to the effectiveness of plants in traditional medicine and other applications [9, 10], and these compounds can be categorized into two major classes, primary and secondary metabolites [11]. Sugars, amino acids, and lipids, are primary metabolites that play a vital role in the basic physiological processes of plants, like growth, development and reproduction [12]. Unlike primary metabolites, such as carbohydrates, lipids, and proteins, secondary metabolites, including alkaloids, flavonoids, terpenoids, and phenolic compounds, are not directly involved in these processes but have been shown to have important roles in plant defence systems, the attraction of pollinators, and communication with other organisms [13]. Secondary metabolites have variable biological activities and pharmacological potential that are investigated in phytochemical studies [14].

Extraction of Phytochemicals from Plants:Extraction of Phytochemicals from Plants:Extraction of Phytochemicals from Plants:Extraction of Phytochemicals from Plants:Extraction of Phytochemicals from Plants:Extraction of Phytochemicals from Plants:Extracted Phytochemicals from Plants:Extraction of Phytochemicals from Plants:Extraction of Phytochemicals from Plants:Extraction of Phytochemicals from Plants:Extraction of Phytochemicals from Plants:Extraction of Phytochemicals from Plants:Plants are some of the most important sources of bioactive and medicinal phytochemicals ergenced. This process includes the separation of bioactive compounds from plant material matrix with the compatible solvents and extraction techniques like maceration, Soxhlet extraction, and supercritical liquid extraction [15]. In extraction, the visited parameters play a palay role in deciding the yield and content of the extracted compounds from the plant species [16] and are not blanket across the different plant species. Some of the methods for analyzing phytochemicals identified from the extracted compounds are chromatography, spectroscopy, and mass spectrometry [17]. This information is important for therapeutic application of the plant and standardization of extracts for medical and other industrial uses [18].

Onopordum cyrenaicum Maire et Wielle among the 50 species Onopordum is one of the interesting species of this genus due to its unique morphological properties and ecological roles. This species originates from Libya and grows well in dry and semi-arid ecosystems, a key to maintaining these natural ecosystems, stopping soil erosion, and creating habitats for organisms [19]. Moreover, Onopordum cyrenaicum has historical importance in Libya, where this species has been utilized for traditional medicine, as well as forage for livestock [20]. Even though it is significant, few scientific studies have been conducted to investigate the phytochemical composition and biological activities related to Onopordum cyrenaicum indicating the need for further studies [21].

This study aims to investigate the phytochemical profile of Onopordum cyrenaicum Maire & Weille, and assess the potential therapeutic uses of this species. Analyzing and characterizing the bioactive substances in this species can contribute to an understanding of the medicinal properties of Onopordum species and lay a foundation for further research on this plant, which is not frequently studiedaq

Experimental

Plant material

A sample of Onopordum cyrenaicum Maire & Weille weighted 2 kg was collected from the valley of Souf El Jeen in Bani Waleed city, during 2024. The botanical identification was performed at the Department of Botany, Bani Waleed University, Libya. The plant sample was cleaned under running tap water and then by distilled water [17]. The cleaned plant sample was dried at 38 ◦C in the shade for 14 days, then

the dried sample were crushed in a blender to gain a powder sample [18]. Finally, the powder of Onopordum cyrenaicum Maire & Weille sample was stored at -4 ◦C for further analysis.

Extraction of the plant material

The powdered plant sample was extracted using a classic process called maceration. 20 g of preserved plant material and 200 ml of petroleum ether were mixed at room temperature (around 40°C in the summer). The mixture was manually shaken for three minutes to ensure homogenization. The mixture stirred for 48 hours then filtered and repeated three times in order to improve extraction. The plant material which had been filtered was further extracted by chloroform 3 × 200 ml and ethanol individually. Eventually, a rotary evaporator was used to remove the extraction solvents, yielding a dry extract which then stored at -4°C for upcoming investigation.

Phytochemical analysis

Qualitative methods were employed to identify the phytochemical components found in Onopordum cyrenaicum Maire & Weille. The procedures were described in reference [19].

Quantifying the total phenols content

The quantity of phenol in the crude extracts were measured by Folin-Ciocalteu reagent technique with a slight modification. Different concentrations (100, 200. 400, 600 µg/ml) of pyrogallol solution were used as a standard solution. 1.5 ml of 10% FCR and 1 ml of 2% of Na2CO3 were added to 0.5 ml of each standard solution separately, and kept for 20 minutes at 40 °C. Finally, a JASCO UV-VIS equipment was used to measure the samples' absorbance at 760 nm. This process was repeated to each crude extract. The TPC of the extracts were reported as mg pyrogallol per g of dried plant [20].

Quantifying the total flavonoid content

The quantity of phenol in the crude extracts were measured by using a slightly modified aluminum chloride method. Several concentrations of quercetin (100, 200, 400, and 600 µg/ml) were prepared as standard solutions. 1 ml of each standard was placed in a 10 ml conical flask containing 0.2 ml of 5% $NaNO_2$ and mixed for 2 minutes. 0.2 ml of a 10% $AlCl_3$ was added to the obtained mixture and mixed for 2 more minutes. Then, 0.5 ml of 0.1 M CH_3COOK was added to the flask, then the volume was continued to 10 ml with DI water and kept away for 20 minutes at 40 °C. Finally, a JASCO UV-VIS equipment was used to measure the samples' absorbance at 415 nm. The TFC of the extracts were reported as mg quercetin per g of dried plant sample [21].

Antibacterial Activity Testing

The bacteria used in this study were S. epidermidis, S. aureus, and MRSA. These bacterial strains were carefully gained from Tripoli Teaching Hospital in Tripoli, Libya. The bacteria were incubated at 37°C for 24 hours to provide the ideal conditions for their culture to grow [22].

Disk Diffusion Method

Bacterial cultures were standardized to the 0.5 McFarland scale and evenly spread onto Mueller-Hinton agar plates using sterile swabs. After a 15-minute drying period, the plates were prepared for sensitivity testing. Disks, 6 mm in diameter, saturated with the respective extracts, were placed on the agar surface. Additionally, Klacid antibiotic was used as a positive control. The plates, arranged with wells spaced 5 cm apart, were incubated at 37°C for 24 hours. Subsequent to the incubation, the plates were inspected for the occurrence of clear inhibition zones around the wells, which were measured to determine the antibacterial effect.

Statistical analyses

The measurements were done in triplicate and reported as the mean ± SD, and the obtained data was analyzed using one-way ANOVA in Excel 2019. The correlation coefficients (R) between TPC and TFC were computed to assess their relationship.

Results and Discussion

Phytochemical analysis

Table 1 demonstrates the phytochemical investigation of Onopordum cyrenaicum Maire & Weiller extracts. The results exposed the existence of bioactive components among the three extracts. The methanol extract indicates the presence of tannins and flavonoids, although saponins, alkaloids,

glycosides, and terpenoids were absent. Ethanol extract revealed the attendance of all the phytochemicals, although petroleum ether extract indicated only terpenoids.

Table 1: Summary of Phytochemical constituents of onopordum cyrenaicum maire & weille extracts.

Extracts	*Alkaloids*	*Glycosides*	*Flavonoids*	*Saponins*	*Tannins*	*Terpenoids*	*TPC (mg pyrogallol /g)*	*TFC (mg quercetin /g)*
petroleum ether	-	-	-	-	-	+	11.21	5.20
Ethanol	+	+	+	+	+	+	78.56	38.63
Chloroform	-	-	+	-	+	-	27.63	16.41

The investigation of the plant extracts showed the existence of a variety of phytochemicals that are identified as clinically and physiologically active. Such as tannins, which are polyphenolic substances known for their antibacterial properties [23]. Flavonoids have a polyphenolic structure with hydroxyl groups, typically produced by plants to deal with microbial infections [24]. Their effectiveness depending on the capability of creating complexes with bacterial cell walls. Terpenoids have antibacterial activity due to their aromatic qualities [25]. Saponins have been shown to inhibit the growth of the gram-positive bacterium, S. aureus [26]. Consequently, the phytochemical analysis indicated that the ethanol extract contains chemical compounds with antibacterial properties, which may explain the results observed in the antibacterial investigation.

Moreover, the ethanol extract indicated the highest TPC value of 78.56 mg GAE/g, while the lowest value of TPC was found in the petroleum ether extract with 11.21 mg GAE/g, despite the fact that the chloroform extract contained 27.63 GAE/g. Nevertheless, the TFC of the OCMW extracts was similar to the latter, with the highest value in ethanol extract (38.63 mg QE/g) followed by the chloroform extract (16.41 mg QE/g), while petroleum ether extract contains the lowest TFC with 5.20 mg QE/g.

Antibacterial Activity

In the current study, three different extracts of Onopordum cyrenaicum Maire & Weille were tested to determine their inhibitory effect against standard bacteria, MRSA, S. aureus, and S. epidermidis. The results in Table 2 demonstrated that the petroleum ether and the chloroform extracts showed no antibacterial activity against the bacteria tested. In contrast, the notable effectiveness of the plant ethanol extract against all bacterial strains emphasizes its potential as a natural substitute for traditional antibiotics. Specifically, the extract exhibited strong inhibitory activity against MRSA, achieving an inhibition zone of 22 mm and 50 mg/ml (MIC).

Table 2: Inhibition zones in mm for Onopordum Cyrenaicum Maire & Weille extracts.

Bacterial types	**Extracts inhibitory zones (mm)**			**Antibiotic (Klacid)**	**MIC (mg/ml)**
	petroleum ether	**Chloroform**	**Ethanol**		
S. aureus (MRSA)	-	03	22	30	50
S. epidermidis	-	06	30	32	3.75
S. aureus	-	06	28	32	6.25

Its activity was even more pronounced against non-resistant S. aureus and S. epidermidis, with a higher inhibition zone and lower MIC value (28 mm inhibition zone and an MIC of 6.25 mg/ml for the former, while 30 mm inhibition zone and an MIC of 3.75 mg/ml for the latter). The activity of the ethanol extract is because of the high concentrations of flavonoids and terpenoids, as revealed by the first phytochemical analysis, which indicates that the bioactive compounds of Onopordum cyrenaicum Maire & Weille may employ various mechanisms of action, potentially targeting multiple critical pathways in bacterial physiology. The significant activity observed against resistant strains highlights the need of isolating and characterizing the responsible compounds, intending to develop them as innovative natural antibiotics.

Conclusion

The current study reveals through qualitative phytochemical examination that Onopordum cyrenaicum Maire and Weille contain significant phytochemicals including terpenoids, alkaloids, glycosides, flavonoids, saponins, and tannins. Ontogenesis of high bioactive contents with therapeutic significance were expressed in seeds of Onopordum cyrenaicum Maire & Weille species. Onopordum cyrenaicum Maire & Weille is a rich source of phytochemicals, which provides a basis for developing medicines for the treatment of disease. More specifically phenolic and flavonoids compounds that known as a strong

chain- breaking antioxidants may directly contribute to lessen free radicals. The significant antibacterial effect of ethanol extract of Onopordum cyrenaicum Maire & Weille suggests the presence of bioactive compounds, however this study could not identify the bioactive compounds responsible for the antibacterial activity so that this can encourage researchers and pharmacists in the development of antibiotics from this plant.

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