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K.A. Atayalla*, M.A. Acka
Sebha University, Faculty of Science, Department of Chemistry, Sebha, Libya;
*e-mail: khamis.ali.atayalla@gmail.com

NOVEL PHYTOCHEMICAL ANALYSIS OF THE ENDEMIC LIBYAN PLANT "*ONOPORDUM CYRENAICUM* MAIRE & WEILLER" AND ITS ANTIBACTERIAL ACTIVITY

Raw plant extracts are considered the best source for herbal medicines and their derivative products. *Onopordum cyrenaicum* Maire & Weiller, a species within the *Onopordum* genus of the *Asteraceae* family, is a medicinal plant endemic to Libya. This study seeks to explore, for the first time, the phytochemical components and antibacterial properties of this plant. A slightly modified maceration technique was employed to extract the bioactive components successively using three different solvents (petroleum ether, chloroform, and methanol). Phytochemical analysis identified the presence of several phytochemical components, including terpenoids, tannins, glycosides, flavonoids, saponins, and alkaloids. Remarkably, the methanol extract exhibited exceptional antibacterial activity, outperforming the other extracts, with inhibition zones of 28 mm for *S. aureus*, 22 mm for MRSA, and 30 mm for *S. epidermidis*, while the minimum inhibitory concentrations (MIC) were 6.25, 50, and 3.75 mg/ml, respectively.

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

1. INTRODUCTION

Plants have long been known as a provider of food and medicine [1]. Ancient civilizations relied on plants to treat ailments; even today, they remain a cornerstone of traditional medicine [2]. The secret to their efficacy lies in a wide range of chemical compounds known as phytochemicals [3]. *Onopordum cyrenaicum* Maire & Weiller belongs to the genus *Onopordum*, tribe *Cardueae* within the family *Asteraceae* [4]. The genus *Onopordum* (*Asteraceae*), containing approximately 50 species [5], includes perennial spermatophyte plants that are extensively used as a traditional therapies of many human ailments [6]. These plants considered a significant supplier of bioactive compounds with a broad range of therapeutic applications [7]. This species is native to Libya with its unique morphological properties and ecological roles, and contributes considerably to both natural ecosystems and cultural practices as it can be seen in figure (1) [8, 9].

The vital role of plant's efficacy due to the wide range of chemical compounds known as phytochemicals, which, classified generally into primary and secondary metabolites [10, 11]. Extraction of phytochemical components from plants is a method of separating bioactive compounds from inactive material using a suitable solvent and ordinary extraction technique. Although primary metabolites, like sugars and amino acids are essential for the plant's basic functions [12, 13], phytochemical components, such as alkaloids, flavonoids, and terpenoids, play roles in defense, attraction, and communication [14]. Phytochemical analysis aims to identify these compounds, exploring their functions and potential applications in medicine, agriculture, and

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Fig. 1. The important plant areas in Libya

environmental science [15, 16]. Among the 50 species of *Onopordum Cyrenaicum* Maire & Weiller which native to Libya, has not yet been explored before, and that is the aim of this work.

2. EXPERIMENTAL

2.1. Plant material

A sample of *Onopordum cyrenaicum* Maire & Weiller weighted 2 kg was collected from the valley of Souf El Jeen in Bani Waleed city, during 2024. The botanical identification was performed by Dr. Hana M. Abdi 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 & Weiller sample was stored at –4 ◦C for further analysis.

2.2. 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 methanol individually. Eventually, a rotary evaporator was used to remove the extraction solvents, yielding a dry extracts which then stored at –4 °C for upcoming investigation.

2.3. Phytochemical analysis

Qualitative methods were employed to identify the phytochemical components found in *Onopordum cyrenaicum* Maire & Weiller. The procedures was described in ref [19].

2.4. 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 solutions. 1.5 ml of 10% FCR and 1

ml of 2% of Na2CO3 were added to 0.5 ml of the 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].

2.5. Quantifying the total flavonoid content

The quantity of flavonoids 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].

2.6. 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].

2.6.1. 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.

2.7. 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.

3. RESULTS AND DISCUSSION

3.1. Phytochemical analysis

Table 1

Summary of phytochemical constituents of *Onopordum cyrenaicum* Maire & Weiller extracts

Extracts	*Alkaloids*	*Glycosides*	*Flavonoids*	*Saponins*	*Tannins*	*Terpenoids*
petroleum ether	-	-	-	-	-	+
Methanol	+	+	+	+	+	+
Chloroform	-	-	+	-	+	-

(+): existence; (–): nonexistence.

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. Methanol extract revealed the attendance of all the phytochemicals, although petroleum ether extract indicated only terpenoids. 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 methanol extract contains chemical compounds with antibacterial properties, which may explain the results observed in the antibacterial investigation.

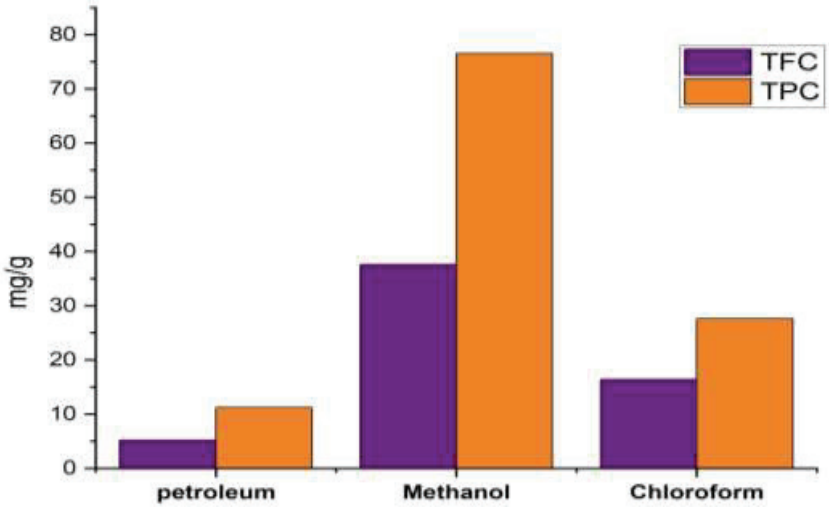

Fig. 2. Illustrates TPC and TFC in the *Onopordum Cyrenaicum* Maire & Weiller extracts

The methanol extract indicated the highest TPC value of 76.56 ± 0.3 mg GAE/g, while the lowest value of TPC was found in the petroleum ether extract with 11.21± 0.1 mg GAE/g, despite the fact that the chloroform extract contained 27.63 ± 0.2 GAE/g. Nevertheless, the TFC of the OCMW extracts was similar to the latter, with the highest value in methanol extract (37.63 ± 0.2 mg QE/g) followed by the chloroform extract (16.41±0.1 mg QE/g), while petroleum ether extract contains the lowest TFC with 5.20 ± 0.3 mg QE/g.

3.2. Antibacterial Activity

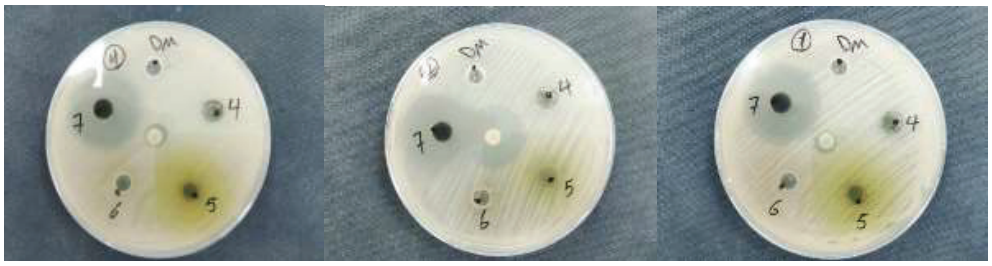

Fig. 3. Shows the inhibition zones of *Onopordum cyrenaicum* Maire & Weiller extracts and Klacid antibiotic (*4, water, 5 petroleum ether, 6 chloroform and 7 methanol*)

Table 2

Inhibition zones in mm for *Onopordum Cyrenaicum* Maire & Weiller extracts

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

In the current study, three different extracts of *Onopordum cyrenaicum* Maire & Weiller 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 methanol 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 ± 0.5 mm and 50 ± 1 mg/ml (MIC).

Its activity was even more pronounced against non-resistant S. aureus and S. epidermidis, with a higher inhibition zone and lower MIC value (28 ± 0.5 mm inhibition zone and an MIC of 6.25 ± 0.1 mg/ml for the former, while 30 ± 0.5 mm inhibition zone and an MIC of 3.75 ± 0.1 mg/ml for the latter). The activity of the methanol 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 & Weiller 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 present work shows that the *Onopordum cyrenaicum* Maire & Weille has some important phytochemicals such as terpenoids, alkaloids, glycosides, flavonoids, saponins, and tannins according to the qualitative phytochemical investigation. *Onopordum cyrenaicum* Maire & Weille species contains a large number of bioactive components with therapeutic importance. The variety of phytochemicals in *Onopordum cyrenaicum* Maire & Weille makes it a potential source for developing medications to treat various ailments. Although the observed antibacterial effect of *Onopordum cyrenaicum* Maire & Weille chloroform extract suggests the presence of bioactive compounds, this study did not identify the specific bioactive compounds responsible for the antibacterial activity, which may encourage researchers and pharmacists to develop antibiotics from this plant.

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К. А. Атаялла*, М.А. Ака
Університет Себха, факультет природничих наук, кафедра хімії, Себха, Лівія;
*e-mail: khamis.ali.atayalla@gmail.com

НОВИЙ ФІТОХІМІЧНИЙ АНАЛІЗ ЕНДЕМІЧНОЇ ЛІВІЙСЬКОЇ РОСЛИНИ «*ONOPORDUM CYRENAICUM* MAIRE & WEILLER» ТА ЇЇ АНТИБАКТЕРІАЛЬНА АКТИВНІСТЬ

Рослинні екстракти вважаються найкращим джерелом для лікарської рослинної сировини та похідних продуктів. *Onopordum cyrenaicum* Maire & Weiller, вид з роду *Onopordum* родини *Asteraceae*, є лікарською рослиною, ендеміком Лівії. Це дослідження мало на меті вперше вивчити фітохімічні компоненти та антибактеріальні властивості даної рослини. Для послідовного вилучення біологічно активних компонентів з використанням трьох різних розчинників (петролейного етеру, хлороформу та метанолу) застосовували модифіковану техніку мацерації. Кількість фенольних сполук в екстрактах визначали з застосуванням реактиву Фоліна-Чокальтеу, результати вказані як кількість мг пірогалолу на грам висушеної рослини. Кількість флавоноїдів в екстрактах вимірювали

за допомогою модифікованого методу з хлоридом алюмінію, результати вказані як кількість мг кверцетину на грам висушеної рослини.
Фітохімічний аналіз виявив наявність ряду біологічно активних компонентів, включаючи терпеноїди, дубильні речовини, глікозиди, флавоноїди, сапоніни та алкалоїди. Слід відмітити, що метанольний екстракт продемонстрував винятково високу антибактеріальну активність, перевершуючи інші екстракти, із зонами інгібування 28 мм для *S. aureus*, 22 мм для MRSA та 30 мм для *S. epidermidis*, тоді як мінімальні інгібуючі концентрації (МІК) становили 6,25, 50 і 3,75 мг/мл відповідно.
Велика кількість біологічно активних речовин у *Onopordum cyrenaicum* Maire & Weille робить його потенційним джерелом в розробці фармацевтичних препаратів для лікування та профілактики різноманітних захворювань. Хоча спостережуваний антибактеріальний ефект досліджених рослинних екстрактів свідчить про наявність в їх складі біологічно активних сполук, це дослідження не виявило конкретних біологічно активних сполук, відповідальних за антибактеріальну дію. Однак значна активність екстрактів, що спостерігається проти резистентних штамів бактерій, підкреслює необхідність виділення та характеристики відповідних індивідуальних сполук, з метою розробки на їх основі інноваційних природних антибіотиків.

Ключові слова: *Onopordum cyrenaicum*, фітохімічні речовини, зона інгібування, антибактеріальні властивості.

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