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Phytochemical Profiling and Assessment of Antibacterial Activity of Linaria Tarhunensis Pamp: A Novel Investigation

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Abstract

Linaria species have been used for a wide range of therapeutic uses. Linaria Tarhunensis Pamp belongs to the genus Linaria, within the family Plantaginaceae, a plant native to Libya that has not been investigated before. The objectives of the current work are to identify the phytochemical, biological effects of Linaria Tarhunensis Pamp extracts using a slightly modified maceration method via two different solvents and to determine its total phenolic contents (TPC) and total flavonoid contents (TFC) employing spectrophotometric methods. Total phenolic compounds were measured using Folin-Ciocalteu reagent and total flavonoids were measured using a quercetin reference standard method. Qualitative screen for the presence of major classes of phytochemical constituent's, alkaloids, flavonoids, phenolics, tannins, saponins, steroids, terpenoids, carbohydrates and glycosides were done according to standard processes. All of the tested compounds were present in the phytochemical analysis except for saponins and tannins. The results indicated that the methanol extract had a high quantity of polyphenols (29.63 mg QE/g) and flavonoids (21.41 mg QE/g), while the chloroform extract contained (11.63 mg QE/g of TFC and 16.56 mg QE/g of TPC). The antibacterial activity of the studied extracts proved ineffective against the used bacterium strains MRSA, S. epidermidis, and S. aureus even though, they might be used as antioxidant or anti-inflammatory.

Keywords: Polyphenols, flavonoids, Linaria Tarhunesis Pamp and antimicrobial.

1. Introduction

Traditional medicine is practiced all throughout the world, especially across Africa and Asia, where it is believed that more than 75% of the population uses plants and their extracts to cure a variety of maladies [1]. The therapeutic value of plants stems from their distinct chemical substances they contain, which have special physiological effects on humans as well as animals [2]. Secondary metabolites derived from plant components are commonly used in many facets of daily life [3] and are known for their extensive biological advantages whether in their raw or processed forms [4, 5]. Due to the existence of bioactive substances in various portions of the plant or the entire plant, medicinal plants have emerged as a key source for enhancing and treating human health around the world [6, 7]. Plants contains a diverse set of chemical substances, including saponins and flavonoids, as well as phenolic compounds or terpenes [8]. Many of these compounds have pharmacological qualities that can be used to build novel therapeutics. Therefore, pharmacologists must have a detailed grasp of these substances and their biological functions. Floristic studies have gained importance in recent years [9, 10]. Driven by the need to quantify plant variety in developed as well as developing nations [11]. Many of these studies have been undertaken globally, concentrating on the flora of various places [12, 13].

Linaria is a member of the Lamiales tribe of the Plantaginaceae family [14, 15], consists of roughly 180 species with a broad distribution across North Africa, Europe and Eastern Asia [16]. Historically, various Linaria species have been employed in traditional treatment for a variety of therapeutic purposes [17]. They have also used in the healing process of infections, hemorrhoids, and vascular problems [18]. These species are widely known as a rich source of numerous bioactive chemicals, including flavonoids, also terpenoids, and alkaloid substances [19, 20]. Although many research investigations focused on the botanical and pharmacological features of Linaria category [21, 22], a limited number of research studies examined the chemical properties of these plants [23]. Notably, Linaria tarhunensis Pamp, a plant distinctive to Libya, is a prime instance of

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a species whose chemical composition and medicinal potential have yet to be studied. This study will be the first to explore the phytochemical composition and the biological potential activity of Linaria tarhunensis Pamp.

2. Materials and Methods

2.1 Plant sample

The Al-Sharshara Valley in Tarhuna city consider the homeland of Linaria tarhunensis Pamp. A sample of Linaria tarhunensis Pamp weighted 2 kg was collected from the Al-Sharshara Valley in Tarhuna city, in June 2024. Dr. Hana M. Abdi at the Department of Botany, Bani Waleed University has identified the plant species. The plant material was washed with running tap water, then rinsed with DI water [24]. The collected plant sample was allowed to dry at 38 ◦C in the shade for 14 days, and then ground into a powder using a blender [25]. Finally, the powder from the Linaria tarhunensis Pamp sample was preserved in an airtight jar for examination.

2.2 Sample Extraction

50 g of preserved plant powder was moved to a 500 ml conical flask holding 250 ml of chloroform and shook for three minutes to achieve homogeneity then left under magnetic stirrer at room temperature (about 40°C in the summer) as part of a slightly modified maceration technique used in this study manually for 48 h. nest step, filtration and repeating the extraction process two times. The filtered plant material was extracted with methanol (3 × 250 ml). Finally, a rotary evaporator was applied to eliminate the extraction solvents, resulting in dry extracts that were kept at -4°C for future research.

2.3 Phytochemical analysis

Qualitative methods were employed to identify the phytochemical components in Linaria tarhunensis Pamp extracts. The procedures described in ref [24].

2.4 Quantifying the Total Phenolic Content

The Folin-Ciocalteu technique was conducted to measure (TPC). In summary, 1.5 mL of FCR (10%) was combined with 0.5 mL of extract. Four minutes later, 1.5 ml of 5% Na2CO3 was added and left in a darkness for 30 minutes. Finally, the combination was tested for optical density at 760 nm [27, 28].

2.5 Quantifying the total flavonoid content

A slightly modified aluminum chloride method was used to quantify the quantity of phenol in the crude extracts. Standard solutions (100, 200, 400, and 600 µg/ml) of quercetin were prepared in different concentrations. Take 1 ml of each standard and mix into a 10 ml conical flask containing 0.2 ml of 5% $NaNO_2$ for 2 min. To the obtained mixture, 0.2 ml of 10% $AlCl_3$ was added and mixed for 2 additional minutes. After that, the 0.5 ml solution of 0.1 M CH_3COOK was injected to the flask, then the volume continued until increased to 10 ml with DI water and it was stayed for 20 minutes at 40 °C. Finally, the sample's absorbance was measured at 415 nm using a JASCO UV-VIS equipment. The total flavonoid contents (TFC) of the extracts were expressed as mg quercetin per g of dried plant sample [27, 29].

2.6 GC-MS Analysis

GC-MS analysis was performed using a Clarus GCMS-QP 2010 Ultra (Perkin Elmer) equipped with an Elite-5MS column (30 m × 0.25 mm ID × 250 µm film thickness). Helium was used as the transportation gas, at the flow rate of 1 mL/min. The oven program began at 70 °C (hold 5 min), then ramped up to 310 °C at 10 °C/min (hold 5 min), resulting in a total run time (50 min). The MS was run in EI mode as follows: interface temperature = 260 °C; ion source temperature = 200 °C; scan spectrum: m/z 40–850; solvent cut time = 4.50 minutes. Samples were injected in a 10:1 split mode at 250 °C.

2.7 Antibacterial Activity Testing

Agar diffusion tests were carried out using S. epidermidis, S. aureus, and MRSA. Paper discs soaked with the extracts were laid out on agar plates containing bacterial strains. The zones of inhibition had been determined after 24 hours of incubation at 37°C [30].

2.8 Statistical analyses

All measurements were carried out in triplicate, calculated as mean ± SD, and obtained data were analyzed in Excel 2019 using one way ANOVA. R values were calculated to assess the relationship between TPC and TFC.

3. Results and discussion

3.1 Phytochemical analysis

Table 1: Phytochemical constituents of Linaria tarhunensis Pamp extracts

Extract	Chloroform	Methanol
Alkaloids	+	-
Glycosides	-	+
Flavonoids	+	+
Saponins	-	-
Tannins	-	-
Terpenoids	+	+
TPC (mg PY /g)	16.56	29.63
TFC (mg QE /g)	11.63	21.41

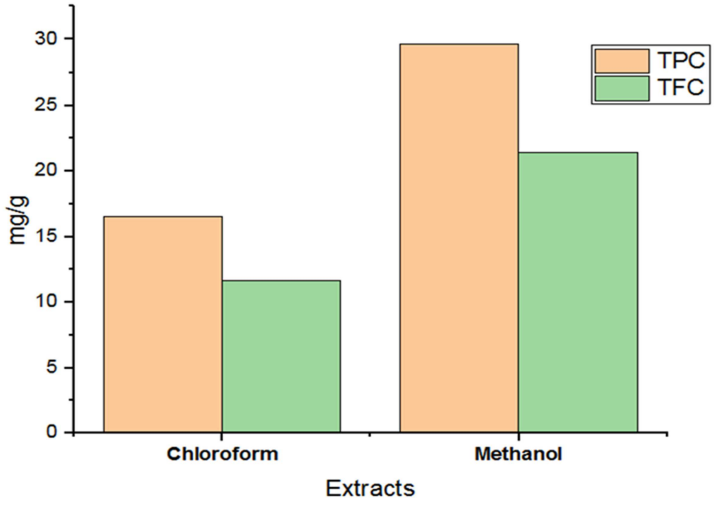

Figure 1: Shows the TFC and TPC values of the extracts.

3.2 Antibacterial Activity

Table 2: Inhibition zones in mm for Linaria tarhunensis Pamp extracts

Bacterial types	Extracts inhibitory zones (mm)	
	Chloroform	Methanol
S. aureus (MRSA)	-	-
S. epidermidis	-	2
S. aureus	-	2

No inhibition zones were seen in the chloroform extract, and the methanol extract had negligible values with 2 mm for S. epidermidis and S. aureus, whereas no inhibition zones were noticed for S. aureus (MRSA), showing that the studied extracts lacked detectable antibacterial activity.

Table 3: Important compounds identified by GC-MS from the extract of Linaria Tarhunensis Pamp

Name of the Component	MW	Formula	R. T
3,7,11- Trimethyl dodeca-1,6,10-triene	206	$C_{15}H_{26}$	*23.10*
ACETIC ACID, 2-CYANO -, 2-(2-CYANO ACETYL)	166	$C_6H_6O_2N_4$	24.73
BICYCLO [3.1.1]HEPT-2-ENE, 2,6-DIMETHYL-6	204	$C_{15}H_{24}$	26.95
URS- 12-EN -28-OL	426	$C_{30}H_{50}O$	29.80
UVAOL	442	$C_{30}H_{50}O_2$	30.13
2R-ACETOXY METHYL- 1,3,3-TRIMETHYL -4T-(3-M	282	$C_{17}H_{30}O_3$	30.51
3-O-ACETYL -6-METHOXY –CYCLO ARTENOL	498	$C_{33}H_{54}O_3$	30.90
LUPEOL	426	$C_{33}H_{54}O_3$	31.26
22ALPHA.-HYDROXY -3,4-SECOSTICT -4(23)-EN	458	$C_{30}H_{50}O$	31.37

The results of this study reveal the significant impact of extraction methods, solvents, and metabolite polarity on the phytochemical constituents of Linaria Tarhunensis Pamp extracts. The varied levels of glycosides, flavonoids, terpenoids, and alkaloids in the chloroform and methanol extracts highlight the crucial role of solvent polarity in affecting the availability and recovery of specific bioactive components. Notably, the methanolic extract had a higher flavonoid content (21.41 mg QE/g) than chloroform extract (11.63 mg QE/g), while The TPC of the Linaria Tarhunensis Pamp extracts was similar to the TFC, with the greatest amount found in the methanol extract (29.63 mg QE/g), whereas the chloroform extract had the lowest TPC (16.56mg QE/g), implying that Linaria Tarhunensis Pamp may serve as a promising source of potential medicinal products targeting oxidative stress and inflammation. However, the relatively low polyphenol content emphasizes the need to investigate if other solvent combinations or extraction procedures could improve the recovery of these chemicals. The absence of saponins and tannins, together with the extracts' poor antibacterial effectiveness, limit the immediate use of L. tarhunensis in antimicrobial therapy. Nonetheless, its high flavonoid and polyphenol content makes it a promising candidate for reducing oxidative damage and inflammation. These characteristics call for additional exploration into its pharmacological features, such as antioxidant and anti-inflammatory activity, in order to determine its medicinal potential.

4. Conclusion

This study provides the first insight into the phytochemical diversity of Linaria tarhunensis Pamp and its potential as a source of bioactive chemicals for the first time. However, the findings highlight the importance of optimizing extraction procedures and investigating alternate solvent systems in order to enhance the recovery of its bioactive constituents. Future research should aim to address these limitations and widen the breadth of its pharmacological examination, perhaps unlocking its true therapeutic efficacy.

5. Conflicts of interest

The authors declare no conflicts of interest related to this work.

6. Acknowledgments

The authors have no acknowledgments to declare.

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