Foods and Raw Materials. 2025;13(2)

ISSN 2308-4057 (Print)
ISSN 2310-9599 (Online)

Research Article
Open Access

Available online at http://jfrm.ru/en
https://doi.org/10.21603/2308-4057-2025-2-647
https://elibrary.ru/YJBDUQ

Barnûf leaves: antioxidant, antimicrobial, antidiabetic, anti-obesity, antithyroid, and anticancer properties

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Received 09.11.2023; Revised 15.02.2024; Accepted 05.03.2024; Published online 02.11.2024

Abstract:
Barnûf (*Pluchea dioscoridis* L.) is a wild plant that grows in Egypt. Barnûf leaves are utilized as a folk medicine, as well as part of food and drink formulations. Their numerous biological benefits include anti-inflammatory and antioxidant properties.
We examined the antioxidant, antidiabetic, anti-obesity, antithyroid, and anticancer activities of methanol, ethanol, and acetone extracts of barnûf leaves.
The methanol extract exhibited the highest total phenolic (241.50 ± 3.71 mg GAE/g extract) and flavonoid (256.18 ± 3.19 mg QE/g extract) contents. All three extracts proved to possess good antioxidant, antimicrobial, antidiabetic, anti-obesity, antithyroid, and anticancer activities. Ellagic acid was the most abundant phenolic acid in the methanolic (30.33%) and ethanolic (24.71%) extracts. The antioxidant experiments revealed that the methanolic extract had potent DPPH$^{\bullet}$ (IC_{50} = 18.21 µg/mL) and ABTS$^{\bullet+}$ (IC_{50} = 17.6 µg/mL) scavenging properties. The acetone extract demonstrated the highest antimicrobial activity against gram-negative bacteria. Regarding α-amylase and α-glucosidase inhibition, the methanolic extract showed the most potent activity with IC_{50} values of 104.28 ± 1.97 and 133.76 ± 2.09 µg/mL, respectively. The methanolic extract also proved to be the strongest inhibitor of lipase and thyroid peroxidase, with IC_{50} values of 127.35 and 211.2 µg/mL, respectively. In addition, the methanolic extract showed the strongest anticancer activity against MCF7-1 and H1299-1 lines with IC_{50} values of 29.3 and 18.4 µg/mL, respectively.
The findings suggest that barnûf leaf extracts could be used in functional foods and pharmaceuticals.

Keywords: *Pluchea dioscoridis* L., medicinal properties, extraction, DPPH, ABTS, herbal medicine

Please cite this article in press as: Essa RY, Elsebaie EM, Abdelrhman WM, Badr MR. Barnûf leaves: antioxidant, antimicrobial, antidiabetic, anti-obesity, antithyroid, and anticancer properties. Foods and Raw Materials. 2025;13(2):394–408. https://doi.org/10.21603/2308-4057-2025-2-647

INTRODUCTION

People have used herbal medicine since ancient times. Seeds, roots, bark, flowers, and leaves of many plants are known to possess medicinal properties.

Although synthetic drugs are quite efficient against a wide range of diseases, they often produce side effects. As a result, herbal medicine has grown in popularity in the last few decades [1, 2]. Medicinal herbs are, by definition, sources of phytochemical substances with medicinal properties. In many cases, plants owe their beneficial properties to secondary metabolites, e.g., alkaloids, terpenoids, or phenolics [3].

Barnûf (*Pluchea dioscoridis* L.) is a big evergreen shrub that belongs to the *Asteraceae* family. In the wild, it grows 1–3 m high, with a lot of branches and a rough, hairy surface. Barnûf grows extensively across the Middle East and in the surrounding African countries. According to Shaltout & Slima, this herb is prevalent in Egypt's western desert oases and eastern deserts, in the Nile valley, along the Mediterranean coast, and on the Sinai Peninsula [4]. It proliferates in demolished dwellings, humid environments, along waterways, depressions alongside highways and railroads, on deserted farmlands, solid or liquid wastes, etc. [5].

Food science knows a variety of solvent systems and techniques that optimize the extraction of polyphenols [6, 7]. For instance, Harborne described a well-designed solvent solution that facilitates the best possible extraction of targeted substances without altering their

chemical structure [8]. Liu *et al.* [9] reported that polar solvents could yield better extraction results for polyphenols than non-polar ones. For this reason, acetone, ethanol, and methanol are the organic solvents frequently employed in combination with water to extract plant substances [10]. Methanol (80%) and ethanol (80%) can be used to increase the yield of polyphenols [11]. Aqueous ethanol (80%) was proposed by Wang & Helliwell [12] as a better solvent for polyphenols than methanol and acetone. Other studies promote acetone as a superior solvent for polyphenol extraction or as an alternative to water and chloroform [13].

Thus, polyphenol production depends not only on the physical characteristics of plant materials but also on the type and polarity of the extraction solvent [6]. As of yet, no specific solvent has been advised for efficient plant phenolic extraction [14]. By choosing the optimal solvent, manufacturers can optimize the extraction process because plant extracts vary in quality. Extracts from barnûf leaves are known to demonstrate potent antibacterial properties against some microorganisms and pathogenic bacteria [5, 15]. Historically, the *Pluchea* genus has often been used as a source of hepatoprotectors, antipyretics, muscle relaxants, laxatives, antiinflammatory agents, astringents, nerve tonics, diaphoretics against fevers, etc. These plants are used as part of treatment against lumbago, cachexia, dysuria, dysentery, necrotizing ulcers, hemorrhoids, and leucorrhoea [16]. Uchiyama *et al.* [17] studied *Pluchea* extracts phytochemically, fractionated them, and revealed polyphenolic components, e.g., flavonoids, phenolic acids, phenylpropanoids, tannins, and chalcones, as well as monoterpenes, lignan glycosides, eudesmane-type sesquiterpenoids, and triterpenoids. All these substances render the plants their antioxidant properties and make them natural detoxification agents.

Synergistically, a combination of these components may provide a greater protection than an individual phytoconstituent [18]. All of these substances have indeed been reported to remove free radicals, reduce oxidation stress, and limit the biomolecular oxidation by disrupting the pathogenic interaction cycles that impair human physiological processes. Free radicals in particular produce cell damage and increase the amount of reactive oxygen species, thus causing tissue damage. Reactive oxygen species escape from the mitochondria in a cascade, thus causing oxidative stress. This mechanism has been linked to the development of type 1 diabetes through the death of pancreatic β-cells and type 2 diabetes through insulin opposition. Additionally, insulin insufficiency encourages fatty acid β-oxidation, which increases hydrogen peroxide production. As a result, pancreatic and liver cells are affected by diabetes and suffer from the elevated quantities of reactive oxygen species [19]. Diabetes mellitus is a major health issue that has a negative and permanent effect on individuals, as well as entire families and societies. Over the past three decades, this issue has grown significantly in scope and is expected to affect 439 million elderly patients by 2030 [20]. Due to their tendency to worsen post-prandial hyperglycemia, α-glucosidase and α-amylase inhibitors are now the most indicated therapies for diabetes. The antioxidant properties of phenolic compounds depend on their characteristics as hydrogen donors, reducing agents, metal ion chelators, and protonated hydrogen quenchers [21]. Natural antioxidants may also be used as a possible treatment for type 2 diabetes mellitus as they reduce postprandial hyperglycemia and block α-glucosidase and α-amylase [22].

Around the world, patients with diabetes show an increased risk of developing such chronic health issues as atherosclerosis, obesity, renal failure, and dyslipidemia [23]. New lipase inhibitors obtained from plant extracts can provide new anti-obesity drugs. Actually, several synthetic medications, including acarbose and orlistat, are often used as inhibitors for these enzymes in people with obesity and type 2 diabetes [24, 25]. However, these inhibitors demonstrated a number of negative side effects [26]. As a result, much effort has been expended in reducing the negative side effects of all of these synthetic hypoglycemia and anti-obesity medications, as well as in discovering safer and natural inhibitors of α-amylase and lipase. Medicinal plants possess photochemically active flavonoids and phenolics with potent antioxidant activities. As a result, they are commonly used to treat diabetes and associated complications [27]. These substances are potent inhibitors of α-amylase and lipase [28].

Environmental elements, e.g., pollution and unhealthy diet, may affect thyroid function [29]. The effects of goitrogenic drugs are a popular research subject [30]. The incidence of goiter is higher if dietary iodine deficiency is caused by thyroid function inhibitors [31].

As the global demand for plant extracts keeps increasing, it triggers an indiscriminate consumption of plants with ambiguous chemical and biological properties. Flavonoids are a class of organic substances that are abundantly present in plants and have been linked to a variety of biological and pharmacological actions in recent years. Thyroid peroxidase is a crucial enzyme for the production and processing of thyroid hormones. It is one of the numerous enzymes that flavonoids can block [32].

According to Bray *et al.* [33], cancer will be the leading cause of mortality in the XXI century. Cancer comes in 36 types that can afflict both women and men. No traditional or contemporary cancer treatment has proved flawless [34]. Numerous variables make it crucial to keep looking for innovative anticancer medications. These concerns include medical procedures that might have serious adverse consequences or that could be rather pricey [35]. Medical scientists are looking for less expensive and more biologically secure options [36]. As far as we know, no comprehensive study has been performed on the therapeutic effect of barnûf leaf extracts, especially their anti-diabetic, anti-obesity, antithyroid**,** and anticancer properties. This research featured the efficiency of various solvents in the extraction of polyphenols from barnûf leaves, as well as the *in vitro* antioxidant, antimicrobial, antidiabetic, anti-obesity, antithyroid**,** and anticancer properties of these extracts.

STUDY OBJECTS AND METHODS

Materials. The fresh barnûf (*Pluchea dioscoridis* L.) leaves were procured in March 2019 from an experimental field of the Agriculture Department, Kafrelsheikh University, Egypt. They were identified as such at the Plants Department, Al-Azhar University, Egypt.

The ethanol (80%), methanol (80%), acetone, gallic acid, quercetin, DPPH, ABTS, butylated hydroxyanisole, ciprofloxacin, fluconazole, *α*-glucosidase, *α*-amylase, lipase, thyroid peroxidase, and guaiacol were acquired from Sigma-Aldrich Chemical Co., USA. Every chemical employed in this research was of HPLC quality, with 99.9% purity.

The nutritional agar and potato dextrose agar media were purchased from Difco Lab, USA.

The samples of *Salmonella typhimurium* ATCC23851, *Escherichia coli* ATCC25921, *Staphylococcus aureus* ATCC25920, *Pseudomonas aeruginosa* ATCC25004, and *Candida albicans* ATCC10230 came from the Microbiology Department, Kafrelsheikh University, Egypt.

Preparing barnûf leaves. The barnûf leaves were washed with pure water. After gathering surplus water with white towels, we left the leaves to dry for a day in an oven (Memmert, UF) at 45 ± 3°C. After that, we pulverized them in an FX1000 electrical crusher (Black & Decker, England) and sieved the powder to produce particles of ≈ 70 mesh [5].

Preparing barnûf leaf extracts. The barnûf leaf powder was extracted using methanol (80%), ethanol (80%), and acetone as solvents. The solvents were selected based on primary experiments. We extracted 20 g of the dried leaf powder in three separate batches by macerating them for 24 h at room temperature in 100 mL of ethanol, methanol, or acetone. All extracts were then vacuum-concentrated at 40°C after being filtered using Whatman filter paper (No. 4 Chr, UK). The resulting extracts were stored at 4 ± 1°C for later use.

Quantifying total phenolics and flavonoids. The technique outlined by Waterhouse [37] was used to estimate the total phenolic contents in the barnûf leaf extracts using a UV/Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) at 765 nm and calculated the results as mg gallic acid equivalent (GAE) per 1 g of extract. The flavonoid content was measured using the method of Zhishen *et al.* [38]. Using a UV/Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) at 415 nm and expressed the results as mg of quercetin equivalent (QE) per 1 g of extract.

High-performance liquid chromatography (HPLC) analysis. The barnûf leaf extracts underwent a HPLC analysis in a food chemistry laboratory, National Research Center, Egypt. The phenolic measurements followed the protocol described by Elsebaie & Essa [39] and involved Shimadzu LC-10A HPLC instruments (Kyoto, Japan) .

Antioxidant activity. ***2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity.*** We used the approach outlined by Fki *et al.* [40] to examine the DPPH radical-scavenging impact. We mixed 5 mL of a 0.004% methanol DPPH solution with 50 µL of variously diluted extracts (0–100 µg/mL) in methanol. After 30 min of room temperature incubation, we measured the absorbance at 517 nm and compared the results with the blank. The percentage of DPPH inhibition, %, was calculated using the following Eq. (1):

$$\text{DPPH inhibition} = \left(\frac{A_b - A_s}{A_b}\right)\times 100 \qquad (1)$$

where A_b is the blank absorbance and A_s is the sample absorbance.

By comparing the graph plotting of the inhibition percentage with the extract concentration, we determined the extract concentration that provided 50% inhibition, i.e., IC_{50}. All assays were run in triplicate and used the synthetic antioxidant reagent butylated hydroxyanisol as a positive control.

2,2'-Azinobis-(3-Ethylbenzthiazolin-6-Sulfonic Acid (ABTS) activity. The $ABTS^{\bullet+}$ method to measure the antioxidant activity of the extracts followed the method developed by Sayah *et al.* [41]. First, we mixed a 5 mM solution of ABTS in phosphate buffered saline with pH 7.4. Then, we combined the ABTS stock solution and MnO_2 to create the ABTS radical cation ($ABTS^{\bullet+}$) and filtered it through a polyvinylidene fluoride membrane. Its absorbance was measured in a 1-cm cuvette after diluting it in phosphate buffered saline (pH 7.4) until equilibrium was reached at 30°C. The mix was then stored at 20°C until use. The final absorbance was measured at 734 nm. After that, we combined 0.05 mL of each extract with 3 mL of the $ABTS^{\bullet+}$ solution at a concentration of 0–100 µg/mL. After a violent shaking in an Eppendorf tube, the mix settled in the dark at room temperature for 6 min before the absorbance at 734 nm was measured. Butylated hydroxyanisole served as a positive control while distilled water was applied as a negative control in place of the extract. The results were expressed by the Eq. (2):

$$\%\ \text{inhibition}\ ABTS^{\bullet+} = \left(\frac{A_b - A_s}{A_b}\right)\times 100 \qquad (2)$$

where A_b is the blank absorbance and A_s is the sample absorbance.

Determining antimicrobial activity. We assessed the antibacterial activity of the barnûf leaf extracts both quantitatively and qualitatively. To study the growth inhibition zones, we used the disc diffusion test as described by Elsebaie *et al.* [42]. We placed 100 mL of cultured cell suspension on each plate. The amount corresponded to 0.5 McFarland of the isolate. After that, we filled the agar plate wells with 100 µL of each barnûf leaf extract, i.e., ethanol, methanol, and acetone. In the antibacterial and antifungal tests, 100 µg/mL ciprofloxacin and 100 µg/mL fluconazole served as positive controls while dimethyl sulfoxide served as a negative control. The plates stayed at 25°C for 1 h to enable pre-incubation diffusion, which reduced the impact of time variation. The plates were subsequently re-incubated in a DSI-D laboratory incubator (Taichung, Taiwan) for 24 h

at 37°C for bacterial strains and 28°C for fungal strains. After the incubation, we examined the plates for antibacterial activity by measuring inhibition zones for each sample. To prevent errors, each test was triplicated for every strain.

In vitro* antidiabetic activity. *α-Glucosidase inhibition assay. The *α*-glucosidase inhibition test followed the protocol developed by Ademiluyi & Oboh [43]. 0.1 mol/L of phosphate buffer with pH of 6.9 contained 0.2 mL of barnûf leaf extracts with concentrations ranging from 0 to 500 g/mL and 100 µL of *α*-glucosidase (0.5 mg/mL). It was allowed to settle at 25 ± 2°C for 10 min. Subsequently, we added 5 mmol/L of *p*-nitrophenyl-D-glucopyranoside solution to the phosphate buffer. After 5 min of incubation at 25°C, the reaction mixes were measured for absorbance at 405 nm using a Bruker 301E spectrophotometer (Rheinstetten, Germany). The *α*-glucosidase inhibition, %, was determined as follows:

$$\alpha\text{-glucosidase inhibition} = \left(\frac{A_b - A_s}{A_b}\right)\times 100 \qquad (3)$$

where A_b is the blank absorbance and A_s is the sample absorbance.

α-amylase inhibiting test. The *α*-amylase inhibition test followed the method developed by Telagari & Hullatti [44]. We combined 200 µL of sodium phosphate buffer (0.02 M) with 80 µL of each barnûf leaf extract at various concentrations that ranged from 0 to 500 µg/mL. The *α*-amylase solution (20 µL) was mixed and kept at room temperature for 10 min. After mixing 200 µL of soluble starch, we left it to settle for 1 h. After adding the 3,5-dinitrosalicylic acid reagent (400 µL) and putting it into a boiling water bath for 5 min, we interrupted the enzymatic reaction by cooling it down and adding 15 mL of distilled water. A UV-Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) was used to measure the absorbance at 540 nm and observe the color change. The *α*-amylase inhibition, %, was calculated by the Eq. (4):

$$\alpha\text{-amylase inhibition} = \left(\frac{A_b - A_s}{A_b}\right)\times 100 \qquad (4)$$

where A_b is the blank absorbance and A_s is the sample absorbance.

***In vitro* anti-obesity activity.** Each sample of barnûf leaf extract was dissolved in dimethyl sulfoxide (10%) to yield stock solutions of 500 µg/mL. These solutions were used to create a concentration dilution series of 0–500 µg/mL. Right before the experiment, we prepared a new stock solution of lipase in a Tris-HCl buffer. *p*-Nitrophenyl butyrate served as a substrate at a concentration of 41.8 mg in 4 mL acetonitrile. After that, we combined lipase and barnûf leaf extracts (0.2 mL) from each dilution series to make workable solutions. After diluting these operating solutions to a final volume of 1 mL with Tris-HCl, we incubated them at 37°C for 15 min. After incubation, each test tube received 0.1 mL of *p*-nitrophenyl butyrate solution. At 37°C, the slurry was incubated again for 30 min. Using a Shimadzu UV-1800 UV/Vis spectrophotometer (Kyoto, Japan), we measured hydrolysis of *p*-nitrophenyl butyrate into *p*-nitrophenolate at 410 nm to evaluate the lipase activity [45]. As before, orlistat served as a standard reference chemical. The lipase inhibition, %, was calculated as follows:

$$\text{Lipase inhibition} = \left(\frac{A_b - A_s}{A_b}\right)\times 100 \qquad (5)$$

where A_b is the blank absorbance and A_s is the sample absorbance.

Antithyroid activity. *Preparing thyroid peroxidase.* We used the method published by Jomaa *et al.* [46], with a few adjustments. The thyroid glands of New Zealand rabbits were purchased from a nearby butcher (Kafrelsheikh, Egypt) and kept at –20°C until needed. We homogenized the material using a Philips homogenizer (Minato-ku, Tokyo, Japan) in a solution that contained 2 mM of Tris-HCl, 0.25 M sucrose, 40 mM NaCl, 100 mM KCl, and 10 mM $MgCl_2$. The resulting mass was centrifuged twice at 4000 rpm at 4°C for 15 min, followed by salting out 60% of enzyme protein. The supernatant stayed in an UGH0044N Kiriazi freezer (Cairo, Egypt) at –20°C until utilized for further analysis.

Thyroid peroxidase inhibitory assay. This test, with a few modifications, also followed the procedure developed by Jomaa *et al.* [46]. The measurement was carried out in a cuvette with a light path of 1.0 cm at a wavelength of 470 nm. The test involved a Shimadzu UV-1800 spectrophotometer (Kyoto, Japan). The mix consisted of guaiacol, 0.1 mol/L phosphate buffer, 40 µL pure material solution, 20 mL thyroid peroxidase enzyme, and 50 µL H_2O_2 at pH 7.4. The combination had a total volume of 210 L. The buffer replaced the barnûf leaf extracts at various concentrations (0–500 g/mL) in the sample probe. The absorbance values were taken at 37°C for three minutes every one minute. The following formula was used to determine the thyroid peroxidase inhibitory activity, %:

$$\text{Inhibition} = \left(1 - \frac{\Delta\text{A/min for test}}{\Delta\text{Amin for blank}}\right)\times 100 \qquad (6)$$

where ΔA/min represents the variation in linearity absorbance, minute to minute, of the test samples; and ΔAmin stands for the variation in linear blank absorption, minute to minute, for the blank samples. The interpolation of dose dependent curves yielded the IC_{50} value.

Anticancer activity. MCF7-1 (breast) and H1299-1 (lung) drug cytotoxicity assays arrived from the National Institute of Oncology in Cairo, Egypt. The potential cytotoxicity of the barnûf leaf extracts was examined using the Natural Red Uptake (NRU) test at concentrations ranging from 0 to 50 µg/mL [47].

Statistical analysis. We used the study of variance (ANOVA), the Duncan test, and the SPSS 17.0 software with $p < 0.05$ as significant.

RESULTS AND DISCUSSION

Total phenolics and flavonoids. Table 1 displays the obtained data for total phenolics and flavonoids based on the absorbance values of the different extracts in comparison to the standard solutions of gallic acid and quercetin. The proportion of flavonoids and polyphenols in every extract proved to be high. The methanolic extract contained the highest amount of total phenolics (241.50 ± 3.71 mg GAE/g) and flavonoids (256.18 ± 3.19 mg QE/g), followed by the ethanol extract with 185.15 ± 3.35 mg GAE/g and 194.24 ± 5.02 mg QE/g, and the acetone extract with 123.47 ± 4.15 mg GAE/g and 136.11 ± 2.97 mg QE/g, respectively. These results were found consistent with those reported by Allouche *et al.* [27], who described polar solvents as optimal for polyphenolic extraction. When the extraction solvent polarity rose, the yield of polyphenols extracted also went up. According to Qasim *et al.* [48], methanolic extracts from *Pluchea* leaves contained more polyphenols and flavonoids than ethanolic and acetone extracts.

Identifying polyphenolic acids. Table 2 shows the polyphenolic composition of barnûf leaf extracts measured by high-performance liquid chromatography (HPLC). The ethanol and methanol extracts contained 12 and 13 phenolic compounds, respectively. Ellagic acid, benzoic acid, catechin, pyrogallol, chlorogenic acid, and gallic acid were the major phenolic compounds presented and identified in the methanolic extract. As for the ethanol extract, the most predominant phenolic compounds were represented by ellagic acid, benzoic acid, catechin, chlorogenic acid, and gallic acid. The acetone extract contained 11 phenolic compounds, the major ones being benzoic acid, ellagic acid, salicylic acid, ferulic acid, and catechin. Pyrogallol, colchicine, and chrysin were found in the methanolic extract only whereas catechol was found in the acetone extract only. These results confirmed those reported by Elsebaie & Essa [5], who found 12 phenolic acids in the barnûf leaf ethanolic extract, ellagic acid and benzoic acid being the most abundant ones.

Table 1 Total polyphenols and flavonoids in barnûf leaf extracts

Solvent	Total phenolics, mg GAE/g extract	Total flavonoids, mg QE/g extract
Methanol	241.50 ± 3.71[a]	256.18 ± 3.19[a]
Ethanol	185.15 ± 3.35[b]	194.24 ± 5.02[b]
Acetone	123.47 ± 4.15[c]	136.11 ± 2.97[c]

The data are displayed as mean ± SD

Values followed by different superscripts in each column differed significantly at $p \leq 0.05$

Table 2 Major phenolic compounds, % total, in different barnûf leaf extracts as identified by HPLC

Compounds	Extract type		
	Ethanol	Methanol	Acetone
Gallic acid	6.71	8.13	n.d.
Protocatechuic acid	4.20	6.01	n.d.
Pyrogallol	n.d.	8.24	n.d.
Catechol	n.d.	n.d.	1.06
Chlorogenic acid	8.27	8.20	6.30
p-Coumaric acid	3.76	1.53	1.11
Catechin	15.28	12.80	8.52
Caffeic acid	5.75	4.60	1.96
Vanillic acid	1.90	2.70	n.d.
Ellagic acid	24.71	30.33	22.60
Caffeine	n.d.	0.29	0.86
Salicylic acid	3.91	n.d.	18.22
Ferulic acid	3.72	n.d.	9.34
Cinnamic acid	0.73	n.d.	0.23
B-OH benzoic acid	21.06	15.75	29.80
Colchicine	n.d.	1.40	n.d.
Chrysin	n.d.	0.02	n.d.

n.d. – not detected

Antioxidant activity. Free radicals have recently been implicated in a number of medical conditions, including heart disease, cancer, ageing, cataracts, immune system damage, etc. [49]. Antioxidants slow down the oxidation rate and shield cells from harm. As a result, they can get rid of unstable free radicals [49]. Antioxidant medications are employed to prevent and cure various diseases that are caused by oxidative stress, e.g., diabetes, Alzheimer's disease, atherosclerosis, stroke, and cancer [50, 51]. Herbal remedies have recently become very popular as an alternative to synthetic medicines because they have no side effects and are less expensive [52]. Antioxidant activity can be measured both *in vitro* and *in vivo*, but very few quick and accurate techniques cover a wide range of plant extracts [53, 54]. In this research, we investigated the ability of barnûf leaf extracts to scavenge the steady free radical DPPH and the cation ABTS in order to explore their antioxidant activity.

DPPH is a stable free nitrogen-centered radical. It is commercially available and has a distinctive absorbance at 517 nm [55]. It provides a common method for assessing plant extracts for antioxidant standards and ability to scavenge free radicals. By absorbing hydrogen from a matching donor, the DPPH solution loses its typical dark purple hue and transforms into yellow diphenylpicryl hydrazine [56]. The overall *in vitro* antioxidant activity of plant extracts has extensively been assessed using this scavenging activity as a rapid and trustworthy criterion [57].

The DPPH test has been applied to antioxidant activities of various medicinal plants [58–60]. These studies reported many plant compounds that act as antioxidants. Figure 1a illustrates the DPPH radical scavenging capacity of different barnûf leaf extracts at various doses. All barnûf leaf extracts demonstrated scavenging activity, which became stronger as the extract concentration increased. At 100 µg/mL concentration, the methanolic, ethanolic, and acetone barnûf leaf extracts all showed enhanced DPPH radical scavenging activities of 83.17, 70.43, and 64.12%, respectively. The acetone extract demonstrated reduced action at all levels. Barnûf leaves

had a significant concentration of phytochemicals that probably donated protons and acted as radical scavengers. Similar findings were reported by Saber [61], who used *Pluchea dioscoridis* leaf extracts to scavenge DPPH radicals in a dose-dependent manner [61].

ABTS stands for 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid. It has a radical cation that may exist in its free state without losing stability. The concentration of radicals was calculated at 734 nm. When an antioxidant was added to the solution of the radical, both its amount and its absorbance went down. This decline depended on the antioxidant activity of the test compound, as well as on the time and concentration [62]. A more effective ABTS decolorization test was used by Re *et al.* [63].

Figure 1b shows how well the extracts were able to remove the ABTS cation. The methanolic extract demonstrated significant concentration-dependent ABTS radical cation scavenging activity. At a concentration of 100 µg/mL, the ethanolic, methanolic, and acetone extracts of barnûf leaves had 89.7, 75.9, and 70.94% scavenging action on ABTS, respectively. This response may point to the ability of barnûf leaves to reduce oxidative damage to a few key bodily tissues at the tested amounts [64]. These findings concur with those reported by Vongsak *et al.* [65], who used the same ABTS test in their research. Figure 1c illustrates a comparative analysis of the IC_{50} values. A low IC_{50} value indicated antioxidant activity. In fact, the maximal DPPH radical inhibition value belonged to the methanolic extract (18.21 µg/mL), followed by the ethanolic extract (37.93 µg/mL) and the acetone extract (54.76 µg/mL). Additionally, the methanolic extract also showed the greatest efficiency against the ABTS radical cation (17.6 µg/mL), followed by the ethanolic extract (30.1 µg/mL) and the acetone extract (43.8 µg/mL). For the DPPH and ABTS assays, the butylated hydroxyanisole IC_{50} values were 10.62 and 9.30 µg/mL,respectively. The presence of additional elements in minute amounts or their combination with the primary ingredients may also contribute to the efficiency of the antioxidant. Our results followed the same pattern as those published by Qasim *et al.* [48] and Saber [61].

Antimicrobial activity. Table 3 describes the inhibition zones (mm) to summarize the antibacterial capacity of various barnûf leaf extracts against two gram-negative bacteria (*Escherichia coli* and *Salmonella thyphimu-*

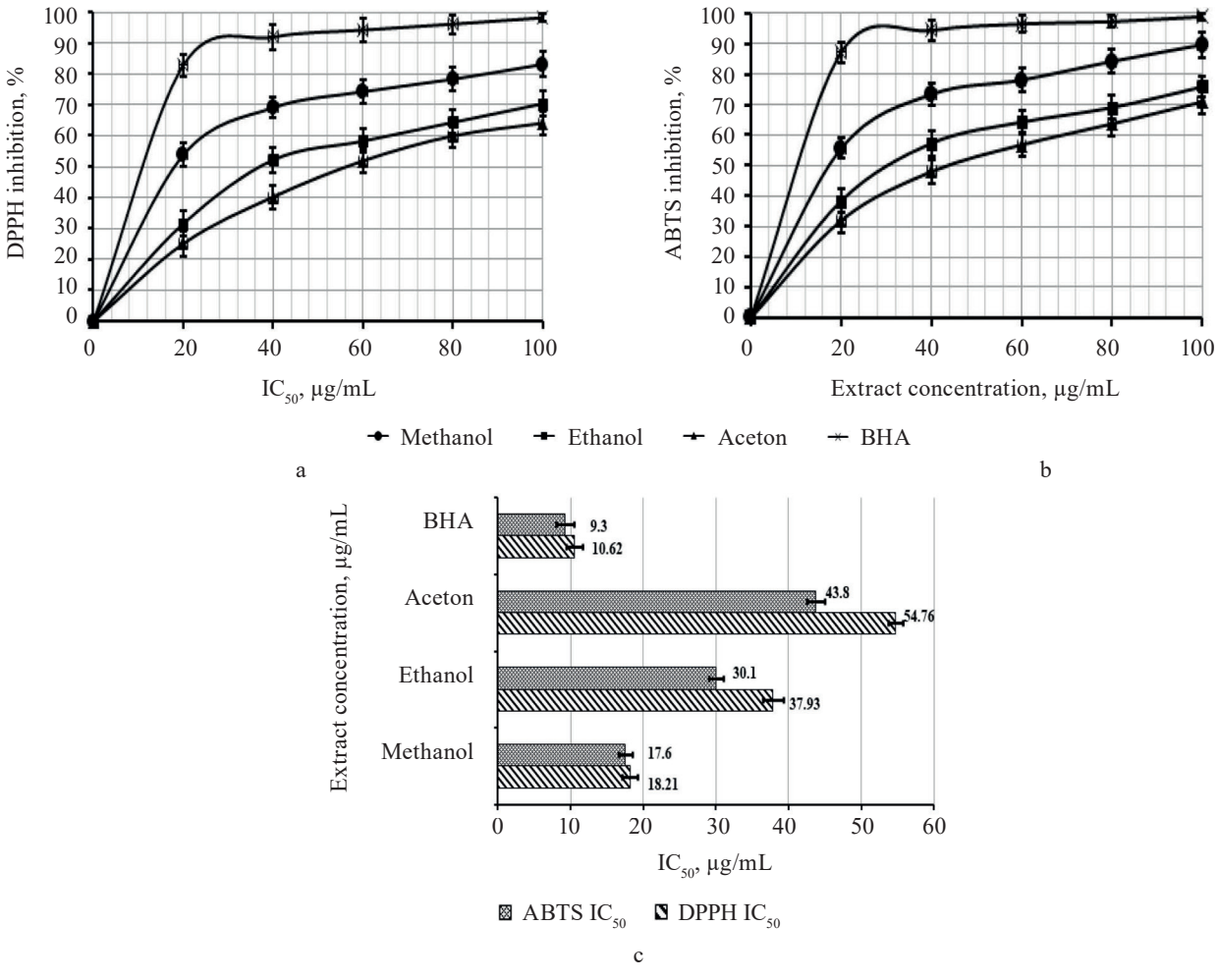


Figure 1 Antioxidant activity of different barnûf leaves extracts by DPPH (a) and ABTS (b); IC_{50} (c)

Table 3 Antimicrobial activity of different Barnûf leaf extracts

Microorganisms	Growth inhibition zone (diameter), mm				
	Barnûf leaf extract			Ciprofloxacin	Fluconazole
	Methanolic	Ethanolic	Acetone		
Escherichia coli	22.3 ± 0.7^{eC}	20.4 ± 0.5^{dD}	24.2 ± 0.3^{eB}	36.1 ± 0.5^{cA}	–
Salmonella typhimurium	24.3 ± 0.5^{dC}	20.1 ± 0.3^{dD}	26.0 ± 0.3^{bB}	40.2 ± 0.8^{aA}	–
Pseudomonas aeruginosa	29.8 ± 0.4^{cB}	26.5 ± 0.7^{cC}	20.6 ± 0.4^{dD}	38.4 ± 0.3^{bA}	–
Staphylococcus aureus	33.2 ± 0.6^{bA}	28.1 ± 0.5^{bB}	23.2 ± 0.5^{eC}	33.3 ± 0.4^{dA}	–
Candida albicans	39.8 ± 0.3^{aA}	35.4 ± 0.6^{aB}	27.5 ± 0.7^{aC}	–	40.2 ± 0.3^{A}

The data are displayed as mean ± SD

Means with different uppercase superscripts (A–D) in the same row are significantly different at $p \leq 0.05$

Means with different lowercase superscripts (a–d) in the same column are significantly different at $p \leq 0.05$.

rium), two gram-positive bacteria (*Pseudomonas aeruginosa* and *Staphylococcus aureus*), and one strain of yeast (*Candida albicans*).

All extracts were obviously effective against the five microbiological strains under analysis. The methanolic and ethanolic barnûf leaf extracts had the highest inhibitory zones against *S. aureus* (33.2 ± 0.6 and 28.1 ± 0.5 mm, respectively). These actions represented 84.38% of ciprofloxacin activity. *E. coli* and *S. typhimurium* were both successfully inhibited by the acetone extract, with inhibition zones of 24.2 ± 0.3 and 26.0 ± 0.3 mm, respectively. The methanolic extract provided larger inhibition zones against *E. coli* and *S. typhimurium* than the ethanolic one but both values were lower than those demonstrated by the acetone extract. These findings are quite significant because the gram-negative bacteria under investigation cause serious intestinal illnesses.

In contrast, the methanolic and ethanolic barnûf leaf extracts were more effective than the acetone extract in killing *P. aeruginosa* and *S. aureus*. Our extracts demonstrated antibacterial efficacy against gram-positive bacteria that was inferior to that of ciprofloxacin. Overall, the acetone extract inhibited gram-negative bacterial strains whereas the methanolic extract inhibited gram-positive bacteria. The obtained results were in line with those obtained by Elsebaie & Essa [5], Al-Salt [66], and Zalabani *et al.* [67]. These results revealed that gram-positive microbes were more sensitive to hydro alcoholic extracts than gram-negative germs, as previously reported by Aruwa *et al.* [68].

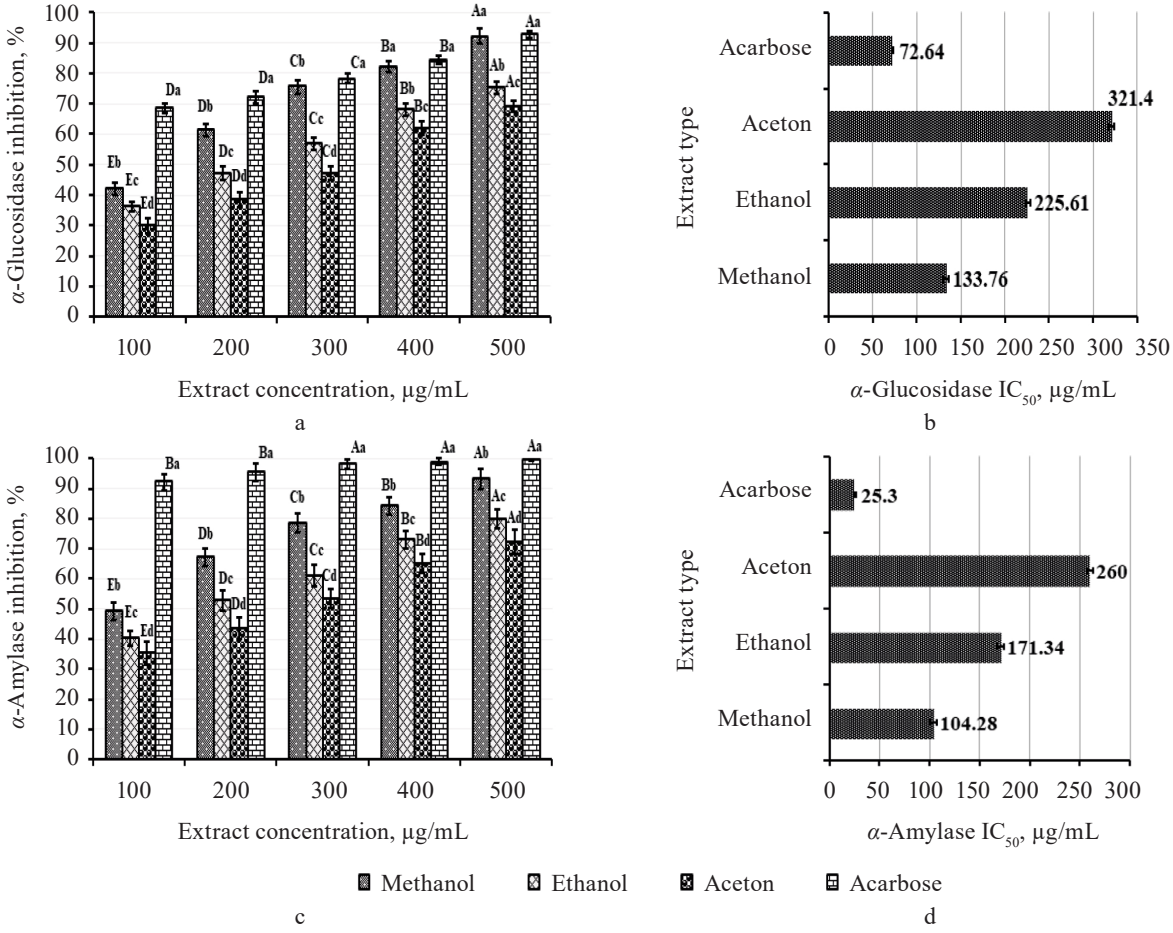
Additionally, the barnûf leaf extracts in methanolic and ethanolic forms were more effective against *C. albicans* than the acetone extract. Our results reconciled with those obtained by El-Ghorab *et al.* [69], who linked the antimicrobial properties of barnûf to its phenolic compounds. Our extracts demonstrated antimicrobial efficacy against all samples, with the exception of the methanolic extract: its activity against *C. albicans* was inferior to fluconazole.

Ciprofloxacin gave larger inhibition zones for *E. coli*, *S. typhimurium*, and *P. aeruginosa* than those obtained by all types of barnûf leaf extracts. The methanolic extract and ciprofloxacin gave similar diameter zones in relation to *S. aureus.* Also, fluconazole and the methanolic extract gave similar diameter zones in relation to *Ca. albicans*, which exceeded those obtained by the ethanolic and acetone extracts. The antimicrobial activity demonstrated by ciprofloxacin and fluconazole against the bacterial and fungal strains in this research was similar to that reported by Elsebaie & Essa [5], Elsebaie *et al.* [42], and El-Ghorab *et al.* [69].

In vitro* antidiabetic activity. *α-Glucosidase inhibition assay. A well-known strategy to combat the metabolic changes caused by type 2 diabetes is to inhibit this enzyme [70]. Generally, *α*-glucosidase inhibitory agents are regarded as oral hypoglycemic medications because they prevent disaccharides from converting into monosaccharides and maintain normal blood sugar levels [19]. We used acarbose, a potent enzyme inhibitor, to compare the results of the *α*-glucosidase test and calculate the IC_{50} values for the three extracts (Fig. 2a and b). The findings show that all barnûf leaf extracts contained potential α-glucosidase inhibitors. Acarbose, which served as reference, had an IC_{50} of 72.64 ± 1.04 µg/mL. The methanolic extract demonstrated the strongest inhibitory effect on α-glucosidase (133.76 ± 2.09). The IC_{50} values for the ethanol and acetone extracts were 225.61 ± 2.97 and 321.40 ± 3.12 µg/mL, respectively, showing only modest *α*-glucosidase inhibition. The variations in phenolic, flavonoid, and antioxidant activities of barnûf leaf extracts may be responsible for this finding. Gowri *et al.* [71] indicated a positive relationship between the total flavonoid and polyphenol contents and the ability to inhibit *α*-glucosidase. These results were similar to ours, as demonstrated in Table 2: the barnûf leaf ethanol and methanol extracts contained 12 and 13 phenolic compounds, respectively.

Error bars represent standard deviation (n = 3). Different lowercase superscripts indicate significant differences at $p \leq 0.05$ between the extracts at the same concentration. Different uppercase superscripts indicate significant differences at $p \leq 0.05$ between the concentrations for the same extract types

α-Amylase inhibition assay. As a major digestive enzyme, pancreatic *α*-amylase is implicated in the decomposition of starch into oligosaccharides before freeing glucose into the bloodstream for absorption. The amount of starch that is broken down in the gastro-



Error bars represent standard deviation (n = 3). Different lowercase superscripts indicate significant differences at $p \leq 0.05$ between the extracts at the same concentration. Different uppercase superscripts indicate significant differences at $p \leq 0.05$ between the concentrations for the same extract types

Figure 2 Effect of different barnûf leaf extracts on α-gluicosidase inhibition (a), α-gluicosidase IC_{50} (b), α-amylase inhibition (c), and α-amylase IC_{50} (d)

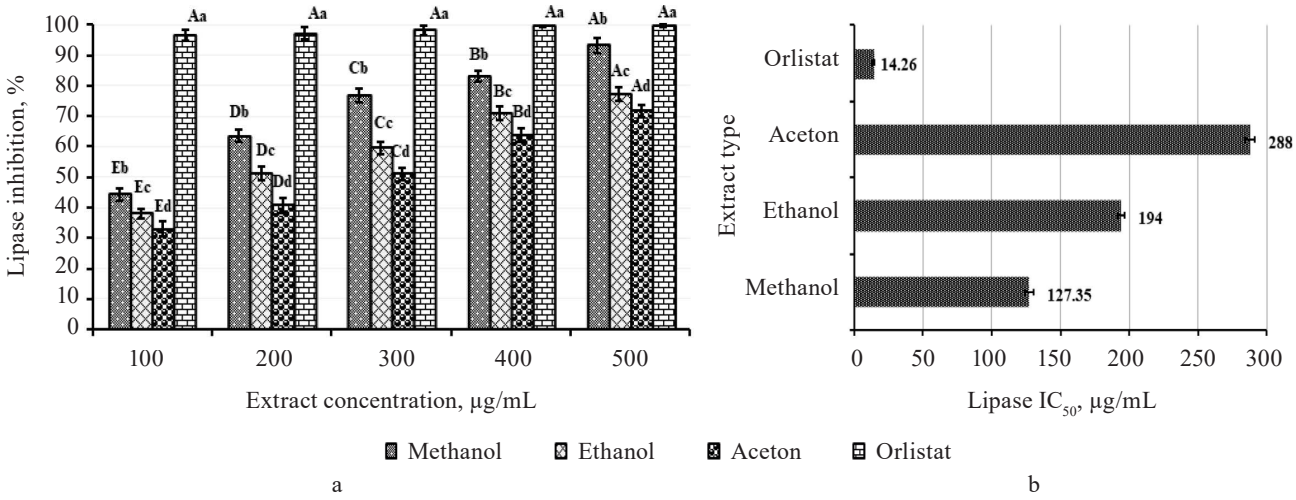
intestinal system would decrease if α-amylase was inhibited. As a result, the amount of hyperglycemia may also be decreased [72]. In this test, we used acarbose, a powerful α-amylase inhibitory drug, to test the barnûf leaf extracts for their anti-amylase effectiveness (Fig. 2c and d). All three barnûf leaf extracts inhibited the α-amylase enzyme in a dose-dependent manner (0–500 µg/mL). The methanolic extract proved to be the most effective α-amylase inhibitor with an IC_{50} of 104.28 ± 1.97 µg/mL, as compared to 25.30 ± 1.62 µg/mL for the reference acarbose. This suggested that barnûf leaf extracts might be an effective herbal treatment against diabetes. The acetone extract exhibited the lowest activity in this assay, with IC_{50} = 260.00 ± 1.97 µg/mL, while the ethanolic extract showed only moderate activity with IC_{50} = 171.34 ± 1.50 µg/mL. Highly polyphenolic herbal extracts demonstrated a stronger potential to block α-amylase, according to Shobana *et al.* [73]. Natural antioxidants and phenolics from plants were reported to possess fewer side effects [74]. The strongest α-amylase inhibitory activity of the methanolic extract may thus be attributed to its high phenolic content and antioxidant capacity.

Our results may be explained by the variation in phenolic, flavonoid, and antioxidant activities of the barnûf leaf extracts. Importantly, some researchers reported a positive relationship between the total flavonoid and polyphenol contents and the ability to inhibit α-glucosidase [71]. These results confirmed our findings presented in Table 2, where the ethanol and methanol extracts contained 12 and 13 phenolic compounds, respectively. Ellagic acid, B-OH benzoic acid, catechin, pyrogallol, chlorogenic acid, and gallic acid were the major phenolic compounds presented and identified in the methanolic extract. Ramkumar *et al.* [75] described ellagic and gallic acids as potent inhibitors of α-glucosidase and α-amylase. The methanolic extract demonstrated the highest content of ellagic and gallic acids, followed by ethanol and acetone. This fact may explain the variations between the inhibitory effects of the three different barnûf leaf extracts against α-glucosidase and α-amylase.

***In vitro* anti-obesity activity.** Lipase is the most crucial digestive enzyme which hydrolyzes dietary lipids into glycerol and fatty acids so that they could be absorbed by the small intestine [76]. As a result, inhibiting this digestive enzyme can help with obesity treat-

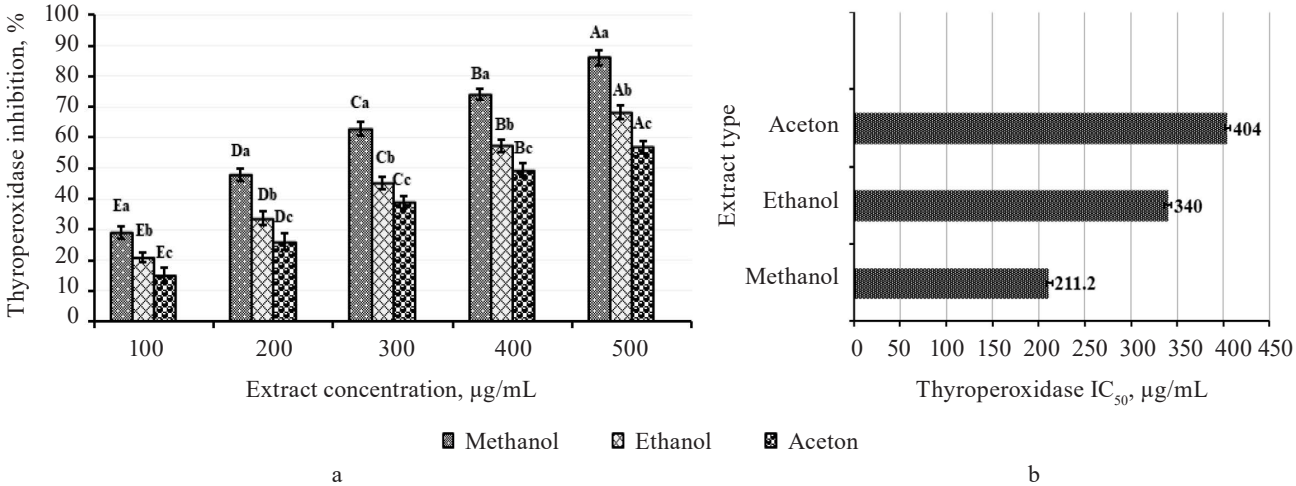
ment [45]. As indicated in Fig. 3a and b all extracts in this research inhibited lipase activity. As a result, the IC_{50} values for methanolic, ethanolic, and acetone extracts against lipase activity were 127.35, 194, and 288 µg/mL, respectively, showing that the barnûf leaf extracts indeed had a potent anti-obesity action. The anti-hyperlipidemic drug orlistat (IC_{50} = 14.26 µg/mL) demonstrated a more powerful suppression of lipase than the other extracts in this research. Additionally, the total phenolics in the various extracts may precisely match their lipase-inhibitory properties. According to McDougall *et al.* [77], the ability to inhibit lipase may come from phenolic components of plant origin, e.g., catechin, gallic acid, epicatechin, myricetin, ellagic acid, kaempferol quercetin, resveratrol, and anthocyanins.

In vitro **anti-thyroid activity.** Thyroperoxidase (EC1.11.1.1-14), commonly known as thyroid peroxidase or iodide peroxidase, is an enzyme involved in the production of thyroid hormones [78]. Since the thyroid peroxidase enzyme is a heme peroxidase, the substrate must first undergo oxidation before it can be oxidized. The H_2O_2 molecule is crucial for its oxidation. The H_2O_2 molecule appears only at the apical surface of thyrocytes, activating any thyroid peroxidase molecules that may be there [79]. Figure 4 displays the thyroperoxidase inhibiting activity of the barnûf leaf extracts. All extracts in this research contained potential thyroperoxidase inhibitors. The methanolic extract demonstrated the most prominent inhibitory activity of 85.89%, followed by the ethanolic and acetone extracts



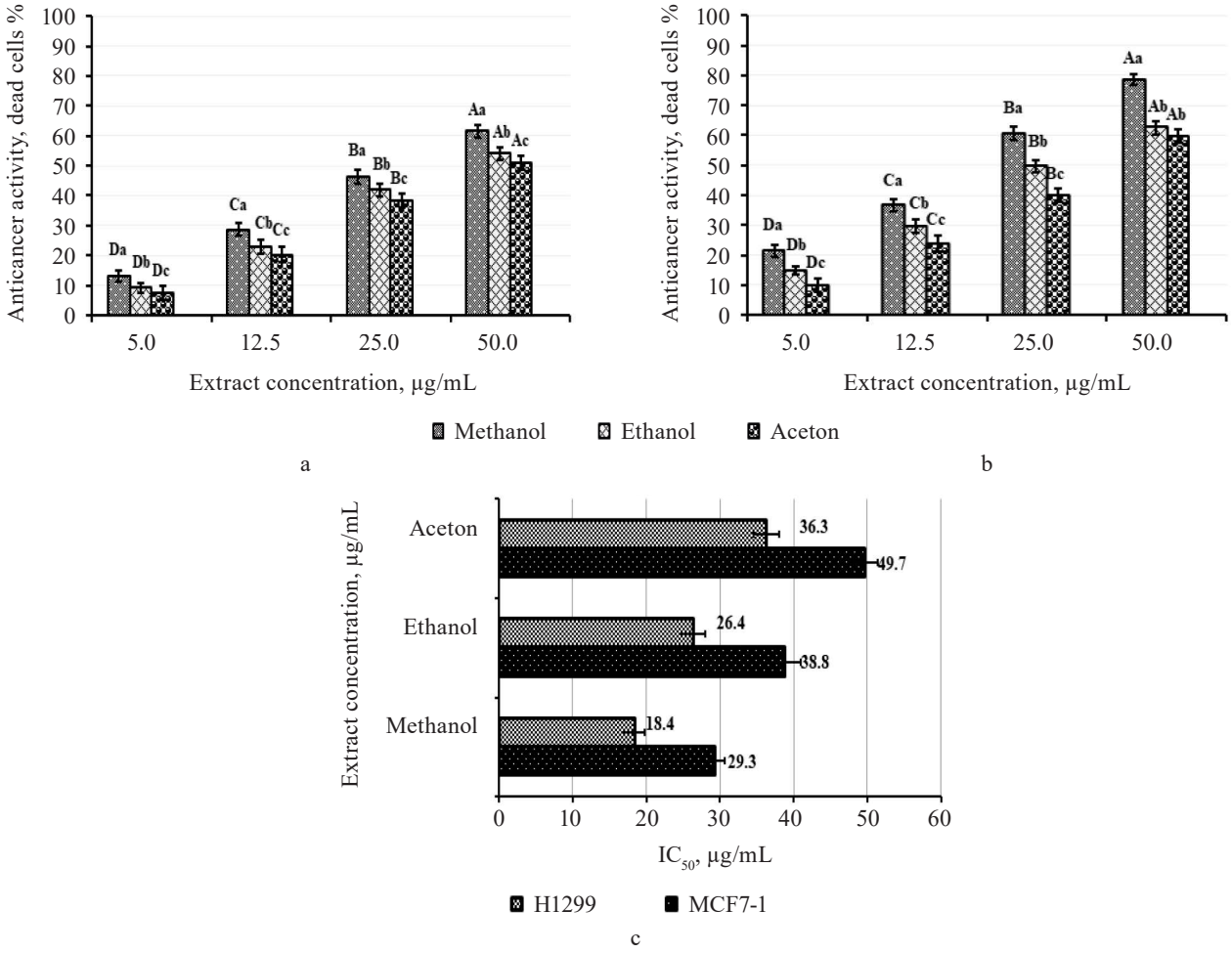
Error bars represent standard deviation (n = 3). Different lowercase superscripts indicate significant differences at $p \leq 0.05$ between the extracts at the same concentration. Different uppercase superscripts indicate significant differences at $p \leq 0.05$ between the concentrations for the same extract types

Figure 3 Effect of different barnûf leaf extracts on lipase inhibition (a) and lipase IC_{50} (b)



Error bars represent standard deviation (n = 3). Different lowercase superscripts indicate significant differences at $p \leq 0.05$ between the extracts at the same concentration. Different uppercase superscripts indicate significant differences at $p \leq 0.05$ between the concentrations for the same extract types

Figure 4 Effect of different barnûf leaf extracts on thyroid peroxidase inhibition (a) and thyroid peroxidase IC_{50} (b)



Error bars represent standard deviation (n = 3). Different lowercase superscripts indicate significant differences at $p \leq 0.05$ between the extracts at the same concentration. Different uppercase superscripts indicate significant differences at $p \leq 0.05$ between the concentrations for the same extract types

Figure 5 Anticancer activity of different barnûf leaf extracts against MCF7-1 (a) and H1299 (b); IC_{50} (c)

in a dose-dependent manner (0–500 µg/mL). Habza-Kowalska *et al.* [78] linked inhibitory properties to the antioxidant activity power. Their results were in line with ours, as illustrated by Fig. 1, where the methanolic extract demonstrated the highest antioxidant activity against DPPH and ABTS, followed by the ethanol and acetone extracts.

The IC_{50} values of the methanol, ethanol, and acetone extracts against thyroperoxidase were 211.2, 340, and 404 µg/mL, respectively (Fig. 4b). Such polyphenolic components as chlorogenic acid, rosmarinic acid, and quercetin were probably responsible for thyroperoxidase inhibition [78].

Anticancer activity. We used MCF7-1 (breast) and H1299-1 (lung) cell lines to assess the potential of barnûf leaf extracts to suppress cell proliferation. Both cancer cell lines were treated with varied concentrations of different extracts. Figure 5 shows that the general activity against H1299-1 was superior to that against MCF7-1. At a concentration of 50 µg/mL, the methanolic extract showed strong anticancer activity against both MCF7-1 and H1299-1 with inhibition percentages of 61.47 and 78.66%, respectively. At the same concentration, the ethanolic extract also demonstrated strong anticancer activity against both lines with inhibition percentages of 54.13 and 62.59%. The acetone extract had a cytotoxic impact on both lines, with inhibition percentages of 50.82 and 59.72% at 50 µg/mL acetone extract concentration. In the methanol sample, MCF7-1 and H1299-1 had IC_{50} values of 29.3 and 18.4 µg/mL, respectively. In the ethanol sample, the IC_{50} values against MCF7-1 and H1299-1 were 38.8 and 26.4 µg/mL, respectively.

Figure 5 demonstrates an inverse relationship between cell viability and sample concentration, with the cell viability percentage declining as the sample concentration rose. The growth of MCF7-1 and H1299-1 cells was negligible, indicating that the barnûf leaf extracts are safe *in vitro* and may be employed as a component in food products, once clinically evaluated on animals and people. The following investigations support the theory that phytochemicals contribute to anticancer properties. Iawsipo *et al.* [80] studied barnûf leaf extracts for anticancer action against breast and cervical cancer cell line. They observed considerable cytotoxicity: the extracts

reduced cancer cell growth even at low doses (15 µg/mL). Bibi *et al*. [81] mentioned about 1000 plant species on Earth as possessing anticancer properties. Our experiment *in vitro* suggests that barnûf is one of these species.

CONCLUSION

The methanolic extract of barnûf (*Pluchea dioscoridis* L.) leaves contained the greatest total phenolics (241.50 ± 3.71 mg GAE/g) and flavonoids (256.18 ± 3.19 mg QE/g), followed by the ethanol extract and the acetone extract. Also, the methanolic extract showed the strongest antioxidant properties against DPPH and ABTS radicals. All barnûf leaf extracts had a potential antimicrobial activity, but the methanolic and ethanolic extracts were more effective than the acetone extract. In addition, gram-positive microbes appeared to be more sensitive to the barnûf leaf extracts than gram-negative bacteria. The extracts demonstrated a powerful suppression of α-glucosidase, α-amylase, lipase, and thyroperoxidase, which suggests that the methanolic extract had good prospects for phytotherapy against diabetes and obesity, as well as an antithyroid agent. Additionally, the methanolic extract inhibited both MCF7-1 and H1299-1 cell lines. These findings might inspire more *in vivo* research to create all-natural pharmaceutical formulations that would be efficient in the treatment of obesity, diabetes, and certain cancers.

CONTRIBUTION

Essam M. Elsebaie was responsible for conceptualization, data curation, formal analysis, investigation, methodology, validation, drafting, review, and editing. Rowida Y. Essa provided data curation, formal analysis, investigation, methodology, software, and the original draft. Wesam M. Abdelrhman was responsible for validation, drafting, review, and editing. Mohamed R. Badr provided data curation, investigation, methodology, software, validation, formal analysis, review, and editing.

CONFLICT OF INTEREST

The authors declared no conflict of interest regarding the publication of this article.

DATA AVAILABILITY STATEMENT

Data available on request due to privacy/ethical restrictions.

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