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Pharmacognostic and Antibacterial Activity of the *Centaurothamnus maximus* leaves

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Abstract

The leaves of *Centaurothamnus maximus* (Forssk) Wagenitz & Dittrich, an endemic Yemeni plant, were evaluated for pharmacognostic and antibacterial activities in this study. Little researches have been done to investigate this plant. Macroscopic and microscopic characteristics were carried out, and physicochemical parameters were established. The 80% methanolic extract of the studied leaves showed antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* in a well-agar diffusion assay, with inhibition zones of 17.66, 17.66, 17 and 17.33, 15.83, 16.83 mm in diameter. These results indicate that the leaves of *Centaurothamnus maximus* could be a useful natural antibacterial agent, and may be used for further study on the plant.

Keywords: Yemeni, *Centaurothamnus maximus*, pharmacognostic, antibacterial

Introduction

Yemen is home to a diverse range of medicinal plants, including *Centaurothamnus maximus* (Forssk) Wagenitz & Dittrich. These healing herbs have received a lot of attention, but not much research has been done on them [1, 2]. In any medical system, authentication and standardization are essential stages [3, 4]. The first step in determining a medicinal plant's identity and purity is to examine it macroscopically and microscopically [5]. Recently there has been a rise in interest in medicinal plants that contain antimicrobial chemicals [6] with fewer side effects and resistance to them [7]. Many phytochemicals have antibacterial properties and can be used to treat various infections [8].

Only Yemen and Saudi Arabia were home to *Centaurothamnus maximus* (Forssk) Wagenitz & Dittrich [9, 10], but few studies have investigated its antibacterial and antioxidant activities [11, 12]. Previous research has found that extracts of these leaves have a significant growth inhibitory effect against human lung cancer, urinary bladder cancer, and breast cancer cell lines, with IC50 values of less than 50 mg/ml, and also have good antioxidant activities at high doses. [13]. No other pharmacognostic, phytochemical, or pharmacological investigations have been done. Therefore the leaves of this plant were selected to evaluate the pharmacognostic properties as well as antibacterial activity as part of our ongoing research on the biological activities of Yemeni medicinal plants.

Materials and methods

Plant material collection and identification

In May 2019, fresh *Centaurothamnus maximus* leaves were harvested in Yafa, Yemen, and dried in the shade before being manually crushed and stored at room temperature for further research. Associate Professor Othman Saeed Alhawshibi of the faculty of Science, University of Aden, Yemen, identified the plant sample.

Plant material extraction

In a Soxhlet apparatus with 300 ml 80% methanol, the air-dried and powdered plant material (30 g) was extracted for 8 hours. After that, the methanolic extract was filtered, evaporated with a rotary evaporator, and stored at 20 °C until required.

Pharmacognostic studies

Macroscopic characteristics

Macroscopic characteristics of the herbal drugs are based on the shape, size, color, surface characteristics, texture, fracture characteristics and appearance of the drugs [5].

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Microscopic characteristics

Freehand sections of the leaves and petiole of *Centaurothamnus maximus* were studied as per the procedure given in WHO guidelines [5]. To make thin leaf pieces transparent, boil them directly on a slide with chloral hydrate solution. Both upper and lower surfaces of the studied leaf were observed under the microscope (Leica USA model 2000 ATC, with ocular: CPL W10X; objective: 4x, 10x, 40x). Trichomes, stomata and epidermal cells were among the distinctive features detected [14]. A digital camera (Sony 10 MP) was used to take the photographs.

Powdered leaf analyses

The studied dry leaves were pulverized into a fine powder. For clarity, a chloral hydrate solution was used. To establish the distinguishing characteristics, a tiny amount of the powder was placed on a slide and examined under a microscope (Leica USA model 2000 ATC, with ocular: CPL W10X; objective: 4x, 10x, 40x). There were several unique characteristics observed [14]. The images were captured using a digital camera (Sony 10 MP).

Physicochemical analysis

The physicochemical parameters (ash values, extractive values and loss on drying) were carried out in dried powder by standard methods as in WHO guidelines [5].

Antimicrobial screening

Source of microorganisms

The following microorganisms: *Staphylococcus aureus* and *Escherichia coli* were used to test the antimicrobial activity. The source of microorganisms, microbial culture and assay were done at the bacteriological laboratory of the Supreme Authority for Medicines in Aden Governorate, Yemen.

Antimicrobial activity assay

According to conventional techniques, the antibacterial activity of the 80% methanolic extract of *Centaurothamnus maximus* leaves was evaluated using a Well-agar diffusion assay [15, 16]. The test bacteria was cultured on nutrient agar plates and incubated at 37°C for 24 hours. Mueller Hinton agar was used with different diluted extract concentrations (10 mg, 7 mg, and 5 mg). Dimethyl sulphoxide solvent was served as a negative control and ciprofloxacin (2.5 µg, 1.75 µg, 1.25 µg) as a positive control. Each test was repeated three times.

Results and Discussion

Macroscopic studies

Pharmacognostic properties are required to confirm the identity and determine the quality and purity of crude drugs [4]. The macroscopic and microscopic characteristics of the studied plant materials are described. The mature leaves are velvety with a green top and white fluff on the bottoms (Figure 1). The leaf is simple, whole, lanceolate, and has a rounded apex. The midrib is big and prominent on the bottom side of the leaf, with prominent veins. The leaf is 3–7 cm wide and 7–20 cm long, with a width of 3–7 cm and a length of 7–20 cm. The leaf is petiolate and has a short petiole (Figure 2).

Microscopic studies of leaves

Epidermal cells with pentagonal, hexagonal, and polygonal shapes were found on both the upper and lower sides of the blade. Anomocytic stomata were seen on both epidermis

surfaces (Figures 3, 4). Quarter and rectangular venation were identified on the blade's upper surface (Figures 5). A cross-section of the blade was made through the midrib, revealing a single epidermis covered in trichomes. Trichomes are uniseriate unicellular. The mesophylls are diffused with palisades (Figure 6). In the midrib of the blade, five open collateral vascular bundles are identified (Figure 7).

The transverse section of the petiole had a general structure that was concave on the adaxial surface and convex on the abaxial surface. Cortex is made up of 4 layers of thin-walled parenchyma cells and 2-3 layers of polygonal angular collenchyma cells. There are five open collaterals in the vascular bundles. Trichomes are uniseriate unicellular trichomes that coat the petiole's epidermis (Figure 8).

Powder microscopy

The study of leaf powder under the microscope showed the presence of a fragment of cells with stomata (Figure 9 A), epidermis with trichomes (Figure 9 B), a group of long simple uniseriate unicellular trichomes (Figure 9 C) and separated thick-walled trichomes with basal cell (Figure 9 D).

Physicochemical analysis

Physicochemical analyses of the studied leaves were performed. Ash value and water content are important in the evaluation of the purity and quality of the drugs. Extractive value is useful to evaluate the chemical constituents present and also helps in the estimation of specific constituents soluble in particular solvents [17, 18]. The results were described in Table 1.

Table 1: Physicochemical parameter of *Centaurothamnus Maximus* leaves

Parameters	Average value (% w/w)
Total ash	8.75%
Acid insoluble ash	0.25%
Water soluble ash	3%
Water soluble extractive value	23%
Ethanol soluble extractive value	13%
Methanol soluble extractive value	15%
Loss on drying at 100-105°C	8.9%



Fig 1: Photo of the flowering top of the studied plant

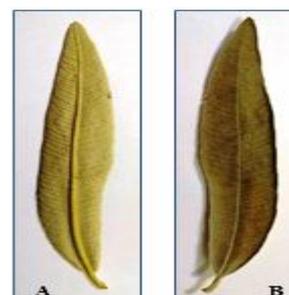


Fig 2: Photo of lower (A) and upper (B) surfaces of the leaf

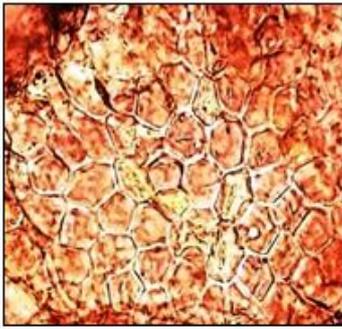


Fig 3: Upper epidermis of the leaf (10x40)

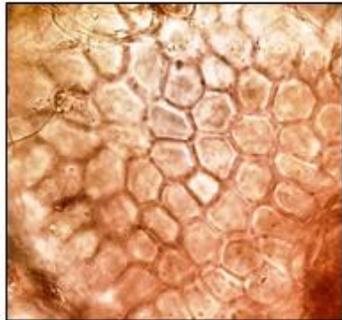


Fig 4: lower epidermis of the leaf (10x40)



Fig 5: Upper surface of the leaf (10x40)



Fig 6: TS of the leaf (10x10)



Fig 7: TS of the midrib (10x40)

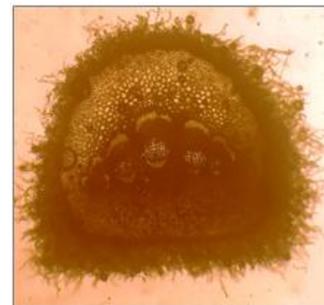


Fig 8: TS of the petiole (10x10)

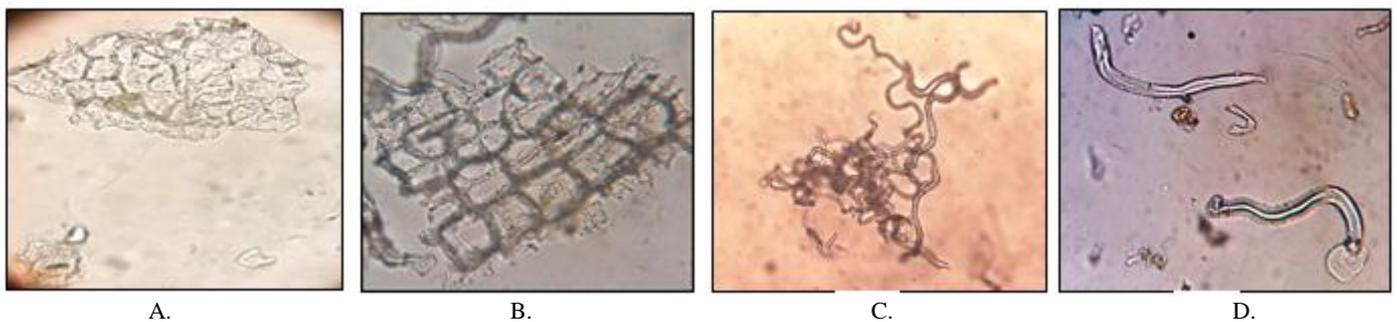


Fig 9: Powder microscopy: fragment of cells with stomata (A), epidermis and trichomes (B), group of long, simple, uniseriate unicellular trichomes (C) and separated thick walled trichomes with basal cell (D) (10x10).

Antimicrobial screening

The antibacterial activity of a methanol 80% extract of *Centaurothamnus maximus* leaves was demonstrated against *staphylococcus aureus* and *E. coli*, with inhibition zones of 17.66, 17.66, 17 and 17.33, 15.83, 16.83 mm, respectively

(Table 2). Plant secondary metabolites have many advantages for the development of anti-infective drugs [19], and they may also act as a vital reservoir of antibiotic adjuvants to overcome resistance mechanisms during treatment [20].

Table 2: Antibacterial activity of 80% methanolic extract of *Centaurothamnus Maximus* leaves

Microorganisms	Zone of inhibition (mm)					
	Methanolic extract			Ciprofloxacin		
	10 mg	7 mg	5mg	2.5µg	1.75µg	1.25µg
staphylococcus aureus	17.66	17.66	17	26	25	24
Escherichia coli	17.33	15.83	16.83	38	35	35

Conclusion

Macroscopic, microscopic and physicochemical parameters have been performed for the leaves of *Centaurothamnus maximus*, and the results will be used to help standardize the drug. The 80% methanolic extract from the *Centaurothamnus maximus* leaves showed antibacterial activity, so they could be used as a prospective drug for pathological infections. However, a more thorough investigation is required to isolate and identify the chemical constituents in addition to the pharmacological activity and toxicological analysis.

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