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ANTIBACTERIAL EFFECT OF ESSENTIAL OIL EXTRACTED FROM *CUPRESSUS MACROCARPA* LEAVES AGAINST SEVERAL BACTERIAL STRAINS

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ABSTRACT: *Cupressus macrocarpa* (*C. macrocarpa*) is an evergreen tree with medicinal uses. The essential oil of *C. macrocarpa* possesses a powerful antimicrobial and antifungal effect against several bacteria and fungi. We aimed of this study to evaluate the antibacterial activity of *C. macrocarpa*. Fresh leaves of *C. macrocarpa* were collected and dried in the shade at room temperature. The essential oil was obtained using hydrodistillation, and yield was recorded. The antibacterial activity of the essential oil against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus vulgaris* was examined, and minimal inhibitory concentration (MIC) was determined using a microdilution assay. As a result, the yield of essential oil from dried and fresh leaves was 0.39% and 0.4%, respectively. The MIC was 0.01 (v/v) for both *Staphylococcus aureus* and *Proteus vulgaris*, where the essential oil exhibited lower activity against *Pseudomonas aeruginosa* with a MIC of 0.04 (v/v). These results show the importance of using *Cupressus macrocarpa* essential oil to treat infection of several known resistant bacteria.

INTRODUCTION: In recent years, bacterial and fungal infections have been exacerbated, and antibiotic-resistant bacterial strains have emerged due to random use of antibiotics. This led to an extensive search for natural sources that have antibacterial activity for possible use as a treatment in medicine and as a preservative in food industry [1]. Therefore, the first trend in our research was toward evergreen plants abundant in our environment, like cypress.

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Cupressus macrocarpa (*C. macrocaroa*) is an evergreen tree up to 23-meters tall with horizontal branches [2]. *Cupressus* has traditionally been used for the treatment of cold, flu, and rheumatism. It is considered a medicinal tree, as its dried leaves are used for stomach pain, as well as to treat diabetes, and its dried fruit is used to treat inflammation, toothache, and laryngitis and as a contraceptive and astringent.

Also, the brunches of Cupressus are used as antiseptic and antispasmodic. The essential oil extracted from *C. macrocaroa* leaves is used to treat rheumatism and whooping cough [3]. The essential oil of *C. macrocarpa* possesses a powerful antimicrobial and antifungal effect against several fungi [4].

MATERIAL AND METHODS:

Plant Collection: Fresh leaves of *C. macrocarpa* were collected in April 2020, from a small forest in southern Corniche, Latakia, Syria. The authentication was performed depending on morphological and microscopic evaluation to detect the plant diagnostic elements. The study was carried out at the department of Pharmacognosy and Department of Microbiology, faculty of pharmacy, Al Sham private University, Latakia, Syria. Five hundrend grams (500 gr) of *C. macrocarpa* leaves were air dried in the shade for two weeks at room temperature 20-25 °C, While 500 grams of leaves were not dried and extracted freshly.

Essential Oil Extraction: EO was obtained using hydro distillation. 500 grams of dried leaves were cut into small pieces, and each 100 grams were mixed with 200 ml of distilled water and extracted using hydrodistillation method for 3 h. The same steps were repeated with the fresh leaves for yield comparison. After 3 hours of boiling, an extract containing essential oil, water and another plants compounds was obtained. The essential oil was separated from the extract using 15 ml of chloroform divided into 3 stages. Then chloroform was evaporated at (70°C), and the yield of essential was recorded [5].

Antibacterial Activity: We studied the antibacterial activity of the essential oil against a Gram-positive strain (*Staphylococcus aureus*) and Gram-negative strains (*Pseudomonas aeruginosa* and *Proteus vulgaris*). These strains were obtained from the laboratory section of Tishreen hospital in Latakia city and maintained on nutrient agar in a temperature of 4°C.

Culture Preparation: *Pseudomonas aeruginosa* strains were isolated from swabs collected from a wide variety of infected wounds and routinely submitted to the Department of Medical Microbiology at Tishreen University Hospital. The isolates were identified as *Pseudomonas aeruginosa* by standard bacteriological techniques. These cultures were maintained by subculture in Mueller-Hinton agar for up to seven days [6].

Antibiotics Sensitivity Test: Antibiotics sensitivity test was performed on bacterial isolates cultured on muller hinton agar media using several antibiotics such as levofloxacin, minocycline, ceftriaxone, cefuroxime, and other antibiotics mentioned in **Table 2**.

Microdilution Assay: One hundred µl of 0.5 McFarland standardized bacterial suspension was added to tubes containing the culture medium and cypress essential oil prepared by double dilution starting from a concentration of 0.04 (v/v) by adding 40 microliter of the essential oil to 1000 microliter nutrient broth and 10 microliter of tween 80 as an emulsifier, then dilution to 0.02, 0.01, 0.005, 0,0025, 0,.0012(v/v).

Control tubes contained only broth (negative control) or bacteria and broth (positive control). Tubes were incubated in the dark at 37 °C for 24 h.

RESULTS:

Essential oil Yield: The yield of essential oil extracted from dried leaves ranged between 0.32 %and 0.46%, and the mean was 0.39%.

We noticed that the color of the essential oil extracted from the dried leaves was darker than the color of the essential oil produced from the fresh leaves, as the percentage of the essential oil extracted from the fresh leaves ranged between 0.34% and 0.48%, and the mean was 0.4%.

Antibacterial Activity: The essential oil has shown antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Proteus vulgaris*, where the MIC was 0.01 (v/v) against both *Staphylococcus aureus* and *Proteus* and against *Pseudomonas aeruginosa*, the MIC was 0.04 (v/v) as mention in **Table 1.**

Antibiotic susceptibility testing indicates that the *P. aeruginosa* isolate was resistant to Nitrofurantoin, cefuroxime and other antibiotics shown in **Table 2.**

TABLE 1: MIC OF *C. MACROCARPA* ESSENTIAL OIL AGAINST STUDIED BACTERIA

Bacterial isolate	0.04	0.02	0.01	0.005	0.0025	0.0012
Pseudomonas aeruginosa	-	+	+	+	+	+
Staphylococcus aureus	-	-	-	+	+	+
Proteus vulgaris	-	-	-	+	+	+

TABLE 2: ANTIBIOTIC SENSITIVE TEST OF PSEUDOMONAS *AERUGINOSA*

Antibiotic Symbol	Antibiotic name	Inhibition zone diameter	Sensitivity
CPR	Cefpirome	40 mm	Sensitive
LEV	Levofloxacin	27 mm	Sensitive
CAR	Carbencillin	20 mm	Sensitive
PPA	Piperacillin	18 mm	Intermediate
POL	Polymyxin	17 mm	Intermediate
MIN	Minocycline	10 mm	Intermediate
CTR	Ceftriaxone	No inhibition zone	Resistant
NIT	Nitrofurantoin	No inhibition zone	Resistant
COT	Colistin	No inhibition zone	Resistant
CXM	Cefuroxime	No inhibition zone	Resistant
CRX	Ceftriaxone	No inhibition zone	Resistant

DISCUSSION: Several studies have been conducted to evaluate natural treatments for bacterial infections.

Here we demonstrate the antibacterial activity of essential oils extracted from *C. macrocarpa* prevalent in the Syrian coast.

We found high activity of *C. macrocarpa* essential oil against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus vulgaris*, making it a good choice for preservative and therapeutic purposes.

The *in-vitro* results of our study provide evidence that *C. macrocarpa* essential oil represents a potentially rich source of antibacterial drugs and food compounds against known resistant bacteria, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.

Our results are similar to a previous Egyptian study where the *C. macrocarpa* essential oil had a lower MIC against gram-positive bacteria, so it is more active against gram-positive bacteria than gram-negative bacteria [7].

Further chemical and Pharmacological examinations of *C. macrocarpa* essential oil are needed to isolate the active chemicals and for additional *in-vitro* and *in-vivo* experiments.

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