In Vitro Inhibitory Effect of *Cyperus rotundus* L Crude Extracts on Mouth Isolates of *Streptococcus mutans* and *Candida albicans*

Najah, A. M.
Department of dental prevention, Technical medical institute, Baghdad, Foundation of technical education

الخلاصة:

السبحيات الفموية عبارة عن بكتريا طبيعية تتواجد في تجويف فم الانسان والتي تعتبر مسببا لتسوس الاسنان والرائحة الكريهة للفم.

يعد هذا البحث دراسة استكشافية للفعالية المثبطة للمستخلص الخام لعشب السعد في نمو جرثومة السبحيات الفموية وخميرة المبيضات البيض بطريقة الانتشار بالحفر.

حضرت ثلاثة تراكيز من المستخلص النباتي (8,16,32 x MIC)، وكان التركيز المثبط الادنى (MIC) للمستخلص الخام (5mg/ml)، وايضا تم اختبار الفعالية التثبيطية للمستخلص الخام وقورن مع المضاد الحياتي الكنامايسين kanamycin.

اظهرت التراكيز الثلاثة للمستخلص تأثيرا عاليا في تثبيط نمو جرثومة السبحيات الفموية قدرها (20.1,23.4,25.2mm) 0ولوحظ اكبر قطر تثبيط كان عند تركيز x 32 مقارنة مع 17,3mm للمضاد الحياتي للكنامايسين kanamycin، كما اشارت النتائج الى وجود تأثير متوسط الفعالية للتراكيز الثلاثة للمستخلص في تثبيط نمو خميرة المبيضات البيض حيث بلغت معدلات اقطار التثبيط (15.3,20.1,21.2mm) لكل تركيز من تراكيز المستخلص، وكان اكبر قطر تثبيط عند تركيز x 32 مقارنة مع 16.7mm للمضاد الفطري amphotreicin B، وهذه النتائج تدعم استخدام العشب لمنع تسوس الاسنان.

Abstract:

*Streptococcus mutan*s is a normal flora bacteria found in human oral cavity, which cause dental caries and bad breathe oder. This project considered as an explorer study for the inhibitory effect of *Cyperus rotundus* L. crude extract on *S. mutans* and *Candida albicans* by agar well diffusion method.

Extract of plant with three concentrations (8,16and 32 fold dilution of the MIC) were prepared and investigated for antibacterial activity. The minimal inhibitory concentration (MIC) against *S. mutans* of crude extract was 5 mg/ml. Crude extract also tested for antimicrobial susceptibility compared with standard antibiotic kanamycin. The three concentrations of the extract showed high effect on the growth of *Streptococcus mutan*s. The means of inhibition zone diameters were 20.1, 23.4, and 25.2 mm for *Streptococcus mutan*s. The largest inhibition zone 25.2 mm in diameter

was observed in crude concentration 32 $_X$ MIC comparing to 17.3 mm in kanamycin. There was moderate effect of the three concentration of extract on the growth of *Candida albicans*, the means of inhibition zone diameters were 15.3, 20.1, and 21.0mm. The largest inhibition zone 21.0 mm in diameter was observed in crude 32 $_x$ MIC comparing to 16.7 mm in amphotreicin B.

Although those results support the traditional use of *Cyperus rotundus* L. for the treatment of dental caries, further study is required for this medicinal plant.

Introduction:

Nature has been a source of medicinal agents for thousands of years and since the beginning of man. Extraction of bioactive compounds from medicinal plants permits the demonstration of their physiological activity. Plants extracts as well as oils are of considerable interest because of their microbial activity [1,2].

Cyperus rotundus L. (family Cyperaceae), also known as purple nutsedge, is a common perennial weed with slender, scaly creeping rhizomes, bulbous at the base and arising singly from the tubers which are about 1-3 cm long. The tubers are externally blackish in color and reddish white inside, with a characteristic odor. The stems grow to about 25 cm tall and the leaves are liner, dark green and grooved on the upper surface. Inflorescences are small, with 2-4 bracts, consisting of tiny flowers with a red-brown husk. The nut is three-angled, oblong-ovate, yellow in color and black when ripe [3,4].

The tuber of this species possess an agreeable aroma and are used for medicinal purposes like, anti-inflammatory, immunomodulator, anti-malarial, anti-viral, antioxidant and hypoglycemic[5,6,7,8].But the inhibitory activity of this weed was not covered well in Iraq.

Among the various oral-micro-organisms, *Streptococcus mutans* has been identified as a plaque-forming bacterium capable of producing dental caries in human[9]. Many attempts have been made to eliminate *S. mutans* from the oral flora. Antibiotics such as kanamycin, chlorhexidine, ampicillin, penicillin, tetracycline, erythromycin and vancomycin are very effective in preventing dental caries *in vivo* and *in vitro*. However their excessive use can result in alterations of the oral and intestinal flora and cause undesirable side effects such as development of bacterial tolerance, vomiting, diarrhea and teeth stains. These problems necessitate further search for natural antimicrobial agents that are save for human and specific for oral pathogens [10,11].

In the last ten years, there has been considerable increase in mycosis and systemic infection caused by *C. albicans* particularly among immunocompromised patients [12]. *Candida albicans* is the most common fungal pathogens isolated from the oral cavity. One of the main causes of oral in candidasis is the presence of grant amount of carbohydrates in the oral cavity [13]. The susceptibility of diabetic patients to cutaneous vaginal and oral candidosis has been well documented [14] and has been linked to the ability of *C. albicans* to adhere to mucous membranes. Studies have

shown an increased resistance of *C. albicans* to azoles such as fluconazole [15] and to amphotericin B [16].This study was designated to evaluate the antibacterial and antifungal activities of *Cyperus rotundus* L.

Material and Methods:

Preparation of plant extract:

A dried roots and rhizomes were partially macerated and 50 gm of it stirrer in 250 ml solvent (ethanol) for 72hr and filtered by gauze then by Wattman no. 1 filter paper. The filtrate was collected and the residue was further extracted with the same solvent, the same procedure was repeated three times. The aqueous extract concentrated by rotary evaporator, the result was gummy mixture and directly was used by adding 1g of mixture to 10 ml of deionized water and brewed as tea by boiling for 15 minutes. The extract was allowed to cool and centrifuged. The supernatant was lyophilized and kept in refrigerator.

In Vitro antimicrobial sensitivity of crude extract:

MICs were determined by the microdilution method recommended by the National Committee for Clinical Laboratory Standards with the Brain heart infusion (BHI) broth. For MIC determinations, suspensions with a turbidity equivalent to that of a 0.5 MacFarland standard were prepared by suspending growth from the (BHI) agar plates in 2ml of sterile broth. Suspension were further diluted to obtain a final inoculums of 5×10^5 CFU per well. The MIC is defined as the lowest concentration of the compound at which the microorganism tested does not demonstrate visible growth. The MIC against *S. mutans* of crude extract was 5 mg/ml. The controls included inoculated growth medium without plant extract sample. Sample blanks contained uninoculated medium only. Trays were incubated in the CO_2 incubator (5% CO_2) at 37C° and the MIC_S were recorded after 24 hours of incubation.

Agar diffusion method:

Agar well diffusion was used to determine the activity of plant extract *in vitro* against *S. mutans* and *C. albicans*. The Mueller-Hinton agar plates were swabbed with inoculums which turbidity equal to a 0.5 McFarland standard. The concentration of crude extract was varied into three concentration which are 8, 16, 32 fold of the MIC. An amount of 300µl of each concentration of crude extract was put in 6 mm diameter cup. All plates were incubated in the CO_2 incubator at 37C° for 24 hours. The diameter of inhibition zone for each concentration after incubation was measured and compared to standard antibiotic (kanamycin) and the negative control (water).

For antifungal activity, two colonies of *C. albicans* were suspended in saline solution. The turbidity was adjusted to equal 0.5 McFarland standard 1×10^5 (CFU/ ml). The suspended cells were swabbed on Mueller-Hinton agar supplemented with 2% glucose. Three wells were made and 300µl of each concentration of plant extract was poured in the wells. The all plates were incubated at 35C° for 48 hours. The diameter of inhibition zone was measured and compared to the standard antifungal (amphotericin B) and the negative control (water).

Results:

The antimicrobial sensitivity of the *C. rotundu*s L. against *S. mutans* was determined by agar diffusion method as described before, the results showed that the three concentrations of extracts plays important role in the clear zone diameters. The increasing of these diameters is related to the increase in extracts concentrations32_X MIC. The largest clear zone of 25.2+0.4 mm was obtained in extract concentration, whereas the positive control kanamycin, produced a clear zone of 17.3 mm. The negative control (water) gave no inhibition zone (Figure1, and Table1). Our result indicated that the extract at the concentration of 32 $_X$ MIC provided the highest inhibitory activity against the *S.mutans*, followed by 16 $_X$ MIC and 8 $_X$ MIC concentrations, followed by 16 $_X$ MIC and 8 $_X$ MIC concentrations, respectively.

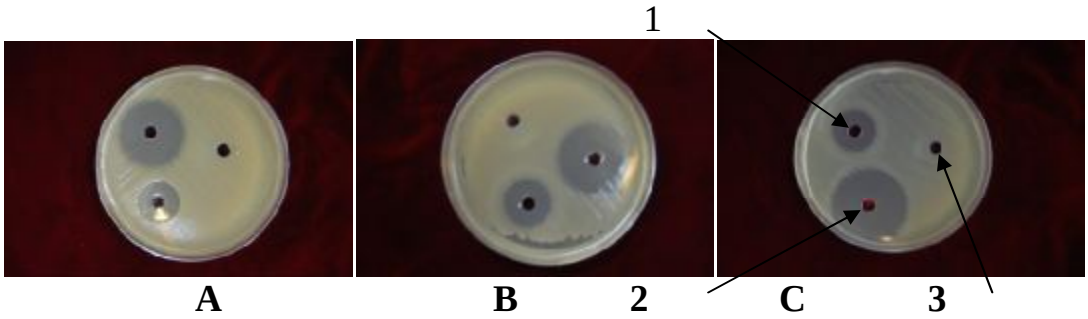

Figure-1: The inhibition zones of crude extract and kanamycin against *S. mutans*: (A) crude extract at 8 $_X$ MIC; (B) crude extract at16 $_X$ MIC; (C) crude extract at 32 $_X$ MIC.
1= positive control. 2= crude extract. 3= negative control

Microorganism	Plant extract concentration $_X$ MIC	Mean inhibition zone of diameters SD
Streptococcus mutans	8	20.1 ± 0.2
	16	23.4 ± 0.3
	32	25.2 ± 0.4
Candida albicans	8	15.8 ± 0.3
	16	20.1 ± 0.2
	32	21.0 ± 0.2

Table-1: The inhibitory effect of crude extract of *C. rotundu*s L. against micro-organisms.

The antimicrobial sensitivity of the *C. rotundu*s L. against *Candida albicans* was determined by agar diffusion method as described before, the results showed that the three concentrations of extracts plays important role in the clear zone diameters. The increasing of these diameters is related to the increase in extracts concentrations32_X MIC.

The largest clear zone of 21.0+0.2 mm was obtained in extract concentration, whereas the positive control amphotericin B, produced a clear zone of 16.3 mm. The negative control (water) gave no inhibition zone (Figure2, and Table1). Our result indicated that the extract at the concentration of 32 $_X$ MIC provided the highest inhibitory activity against the *Candida albicans* followed by 16 $_X$ MIC and 8 $_X$ MIC concentrations, followed by 16 $_X$ MIC and 8 $_X$ MIC concentrations respectively.

Figure-2: The inhibition zones of crude extract and amphotercin B against *C. albicans*: (A) crude extract at8 $_X$ MIC; (B) crude extract at16 $_X$ MIC; (C) crude extract at32 $_X$ MIC.
1= positive control. 2 = crude extract. 3 = negative control.

Discussion:

The crude extract of *C. rotundu*s L. showed antibacterial activity against *S. mutans* and antifungal activity against *C. albicans*. The results showed that the three concentrations of extracts plays important role in the clear zone diameters. The increasing of these diameters is related to the increase in extracts concentrations. The antimicrobial activities of *C. rotundu*s L. has been reported by several authors [15,16,17,18]. The crude extract of *C. rotundu*s -pinene and camphene which are responsible for antimicrobial activity[19]. On the evident that the bioactive compounds such as polyphenols, flavonoids in the *C. rotundu*s L. extract is involved in bacteriostatic activity against *S. mutans* [20], by its ability to form hydrogenous bond with protein which lead to stopping protein built up in the cell[21].

The results of the present study suggest that *C. rotundu*s L. extract had effect on the growth of *S. mutans* and *C.albicans*. Also the results showed that the three concentrations of extracts plays important role in the clear zone diameters. The increasing of these diameters is related to the increase in extracts concentrations The main components of *C. rotundu*s L. are -pinene and 1,8-cineole are due to a change of membrane permeability arising from membrane alteration[22]. The phenol compound of C. *rotundu*s L. could be considered clinically as antifungal agent. The n-hexane fraction of *C. rotundu*s L. exhabits antimicrobial activity against *Streptococcus* species, and *C.albicans* [23].

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