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COMPARISON BETWEEN NANOPARTICLE , PHENOL, AND ALCOHOL EXTRACT OF *CONOCRPOUS* LEAVES ACTIVITY ON SOME PATHOGENIC FUNGI.

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Abstract:

The medicinal plant had been discovered and used in traditional medicine practices since prehistoric times. *Conocarpus* leaves extracts to have anti-fungal antioxidants, antimicrobial and anticancer activities. This research used alcohol , phenol, and AgNPs extract from *Conocarpus* leaves with concentrations 5, 10, 15 mg\ml the results showed that the most fungal affected by both crud and phenol extract was *Cryptococcus neoformans*; while at AgNPs extract highest inhibition zone was at *Milerozyma farinose*. However, the largest inhibition zone was at crud, AgNPs, and phenol extracts successively.

Keywords: antifungal, alcohol and AgNPs extract, *Conocarpus* leaves, Pathoginc fungi .

مقارنة بين تأثير فعالية مستخلص جسيمات الفضة النانوية ومستخلص الفينول والمستخلص الكحولي لأوراق الكونوكاربوس على بعض الفطريات الممرضة

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الخلاصة:

اكتشف النبات الطبي واستخدم في الطب التقليدي منذ عصور ما قبل التاريخ. تحتوي مستخلصات أوراق *Conocarpus* على مضادات الأكسدة المضادة للفطريات ومضادات للميكروبات والسرطان. في هذا البحث تم استخدام مستخلص الكحولي والفينولي ومستخلص جسيمات الفضة النانوية من أوراق *Conocarpus* بتركيز 5 ، 10 ، 15 ملغ / مل ، وأظهرت النتائج أن أكثر الفطريات تأثراً بكل من مستخلص الكحولي والفينول كانت *Cryptococcus neoformans*. بينما في مستخلص AgNPs كانت أعلى منطقة تثبيط في *Milerozyma farinose*. وجد ان أكبر منطقة تثبيط في المستخلص الكحولي ، ثم مستخلص AgNPs ، وثم المستخلص الفينول على التوالي.

الكلمات المفتاحية: مضاد فطري، مستخلص كحولي ونانوي، اوراق الكونوكاربس، فطريات ممرضة.

INTRODUCTIO

Conocarpus erectus; which is known as buttonwood; is an evergreen tree, with (6 m) tall with a spreading crown [1]. *C. erectus* extract from different parts such as (leaves, stems, fruits, and flowers) were showed a high antioxidant, hepatoprotective and anticancer activity because of the presence of the phenolic compounds. It is a try to review the pharmacognostic properties, traditional uses, and biological activities of the plant [2] *Conocarpus* has biological activity against fungi and bacteria [3]. They may have great relevance in the prevention and therapies of diseases in which oxidants are implicated after more in vivo studies [4].

The bioactivity of *Conocarpus* spp. Where comes from (9) compounds of alkaloids, (5)compounds of saponins, and (8) compounds of total phenolic compounds found by thin-layer chromatography (TLC) to C. lancifolius leaf extract [5].

Nanoparticles NPs are those particulate materials that have at least one dimension(1D) that is less than (100nm) [6]. There are many evidence that sight that Ag ions are important in the antimicrobial activity of AgNPs[7, 8, 9, 10, 11].

MATERIAL AND METHODS

leaves of the plant collection

Plant leaves were collected at 26\10\2020 to 25\11\2020 from the gardens of Baghdad city / Iraq was washed more than once with tap water running to get rid of the soil and dust and then dried by the heat 40-45˚C for one day, then leaves of the plant will be blended with the help of blended.

Fungi Isolation

Fungi were used in this study obtain from Baghdad university, collage of science, department of biology.

Preparation of culture media PDA

Potato dextrose agar medium (PDA),with amoxicillin 500 mg was used for fungi growth and antifungal sensitivity test.

Five wells (5 mm) were made on PDA plate with sterile cork borer(well diffusion method) . To each well, different concentrations of test extracts (20,15,10,5) mg/ml were added. Controls were maintained with DW. only. The treated and the controls were kept in an incubator at 37°C for 5 days and inhibition zones were measured. Three replicates were maintained for each treatment [12].

Preparation of alcohol extract

Taken 30 gm of ground leaves of *Conocarpus* erectus with 300 ml of 70% methanol solvent, then put in a soxhlet for 6 hours for alcohol leave extract. The solvent was removed in a vacuum by using a rotator evaporator, to know the weight of each crude methanol extract [13]. Then put the alcohol extract in dishes and put it in the oven at 40°C overnight to dry the extract and take it as a powder [14].

Phenols plant extracts

For phenols preparation from plant

extracts we follow these steps as [15]:

1. Taken 200 g of plant powders divided into two equal quantities; one of them mixed 300 ml D.W., and the other mixed with 300 ml of 1% HCl.
2. Homogenized in an electrical shaker for 5 min.
3. Warmed it for 30-40 min. the supernatant was mixed with N-Propanol and saturated with NaCl in the separated funnel.
4. The upper layer dried in a rotary evaporator at 40°C while the lower layer (aqueous layer), extracted with ethyl acetate and concentrated by using a rotary evaporator at 40°C.
5. At the end dried material for both layers mixed and dissolved with(5 ml) of (96%)ethanol then dried in the oven at 40°C and kept in the refrigerator until uses.

Synthesis of silver nanoparticles

To a prepared aqueous solution (1mM) of silver nitrate (AgNO3), to synthesis AgNPs, (5 ml) of plant extract were added into (5 ml) of this solution of 1 mM AgNO3 for reduction into Ag+ ions. In Erlenmeyer flask and heated on water bath at (75° C) for (60 min) [16].

Characterization of AgNPs:

change in color

The formation of AgNPs was confirmed by a change in the color of the solution [17].

Ultraviolet-visible spectroscopy:

The sample was measured by using UV-VIS double beam spectrophotometers, to ensure the formation of AgNPs at specific rang [18].

Atomic force microscopy

In this research use (AFM), The sample was imaging by AFM- contact mode by Angstrom Advanced [16].

Scanning electron microscope

To characterize the morphological description of the AgNPs sample was done using JEOL Jsm6480 LV for SEM analysis. The samples were scattered on a slide, after that coated with platinum in an auto fine coater, then the material was subjected to analysis [19].

RESULTS AND DISCUSSIONS

Antifungal activities of *Conocarpus* leaves extracts

Antifungal activities of crude methanolic, AgNPs, and phenolic extracts of *Conocarpous* leaves plant were evaluated against fungal pathogens with test sensitive (well diffusion technique).

Crude methanolic extracts

Five pathogenic fungi (*Cladosporium, Candida famata, Cryptococcus neoformans, Milerozyma farinose, Rhodotorula harrison,* and *Candida albicanas*) were used to notice the effect of Conocarpous leaves extract on pathogenic fungi. The result showed that the inhibition effect of these crude methanolic extracts with different concentrations (5, 10,15,20) mg/ml for sensitive test shown in table (1), that *Candida albicanas*, *Rhodotorula harrison*, and *Cladosporium* were the highest sensitive at 5mg\

ml than other fungal. The highest inhibition zone at sensitive test was at concentration 15mg/ml in *Rhodotorula harrisonabout* 23.3mm, C. famata about 1cm in 20mg/ml, and at concentration 5mg/ml in *C. albicanas* about 23.3mm.

The results of this study were identical to that found by[20], who find that *C. erectus* extracts have the most effective on the growth of Aspergilous flavus with inhibition zone (4mm) as radius. The study of [21] found that the extracts of *C. erectus* had extreme effectiveness against the growth of *Alternaria solani* and *Ulocladium botrytis* [21].

Table 1. The inhibition zone (mm) of crud extract effect on pathogenic fungi.

Fungi	Mean ± SE				LSD value
	Conc . 5 mg\ml	Conc . 10 mg\ml	Conc . 15 mg\ml	Conc . 20 mg\ml	
Cladosporium	12.00 ±0.65	19.30 ±0.12	10.7 ±0.47	10.00 ±0.47	0.544 **
Candida famata	5.00 ±0.15	5.66 ±0.08	6.00±0.23	10.00 ±0.28	0.491 *
Cryptococcus neoformans	11.70 ±0.08	8.33 ±0.14	6.00 ±0.00	4.00 ±0.06	0.577 **
Milerozyma farinosa	11.00 ±0.10	7.66 ±0.06	7.00 ±0.11	4.00 ±0.06	0.542 **
Rhodotorula harrison	21.70 ±0.13	22.70 ±0.03	23.3 ±0.08	23.00 ±0.00	0.407 NS
Candida albicanas	23.30 ±0.03	21.70 ±0.03	20.00 ±0.00	16.30 ±0.32	0.566 **
LSD value	0.874 **	0.281 **	0.683 **	0.809 **	---
* (P≤0.05), ** (P≤0.01).					

Phenolic extract

This part of the research studied phenolic extract from leaves of Conocarpous on some pathogenic fungal and measure inhibition zone format. At low phenolic extract concentration, they inhibit growth zone appeared in figure (1). *Candida albicanas* have the largest zone then *Cladosporium, Rhodotorula harrison, Cryptococcus neoformans, Milerozymafarinose*, then *Candida famata* it about (19.00, 14.30, 11.30, 5.66, 5.33, 2.67) mm sequentially at concentration 5mg/ml, (P≤0.01) table (2).

The results of [22] revealed the effect of the *Alhagi maurorum* fungal species phenolic extract, which depend on different concentration of the extract and the fungal species. It found that effect of phenolic extract at (0.25-2 mg/ml) at *Altenaria alternata* (p<0.01).

The research of [23], where they isolated some phenolic substances and found that they had an effect on *Candida albicans* as well as non-albicans species, that one of the effective phenolic compounds by increasing levels of reactive oxygen species (ROS) and inducing early apoptosis[23]. While the phenolic effect on *Cryptococcus*

neoformans studied by [24], and [25] that phenol has Synergistic activity With pharmaceutical antifungals that could be due to either disruption of the cell wall,or by forming lesions in the plasma membrane[24], and [25].

Table 2.The inhibition zone (mm) of phenol Conocarpous extract effect on pathogenic fungi.

Fungi	Mean ± SE				LSD value
	Conc . 5 mg\ml	Conc . 10 mg\ml	Conc . 15 mg\ml	Conc . 20 mg\ml	
Cladosporium	14.30 ±0.53	16.30 ±033	17.00 ±0.47	19.70 ±0.03	0.502 **
Candida famata	2.67 ±0.12	3.33 ±0.03	4.33 ±0.13	5.66 ±0.03	0.315 NS
Cryptococcus neoformans	5.66 ±0.03	7.33 ±0.14	10.70 ±0.06	12.30 ±0.23	0.603 **
Milerozyma farinose	5.33 ±0.17	9.00 ±0.06	9.33 ±0.08	13.70 ±0.18	0.522 **
Rhodotorula harrison	11.3 ±0.27	12.30 ±0.42	13.30 ±0.36	17.70 ±0.13	0.518 **
Candida albicanas	19.00 ±0.26	20.00 ±0.43	22.33 ±0.23	23.30 ±0.08	0.435 NS
LSD value	0.765 **	0.832 **	0.931 **	0.533 **	---
** (P≤0.01).					

Milerozyma farinose

Figure 1. Sensitivity test of phenolic extract of C. leaves on some pathogenic fungi (Milerozyma farinose).

Biosynthesis of silver nanopartilces (AgNPs)

Many indicators have been used to improve AgNPs formation as noted below :

Change in color

The first indicator for AgNPs formation is color change as visual observation of the aqueous extract plus AgNO3 solution from pale brown to dark brown color (figure 2), as a result of AgNO3 oxidation with plant extract partial. Changing in color from pale to dark brown indicates the biosynthesis of AgNPs notes by [26]were synthesis silver nanoparticles from *Cyperus sp. Rhizomes*.

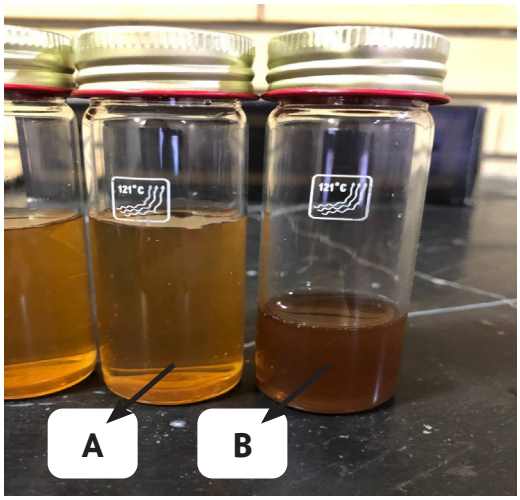
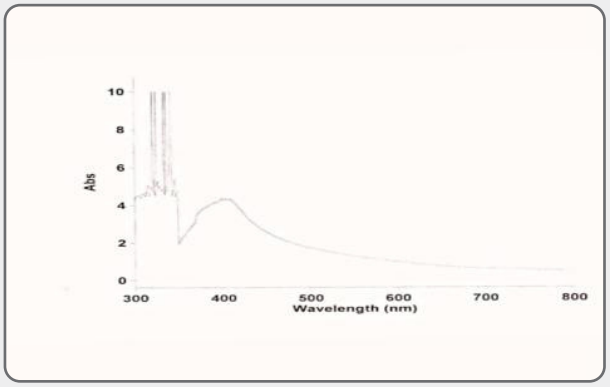


Figure 2. AgNPs color changed, a: before AgNO3 oxidation with plant extract partial, b: after AgNO3 oxidation with plant extract partia.

Ultraviolet-visible spectroscopy:

Formation and stability of prepared AgNPsin sterile distilled water were confirmed by using UV which is a spectrophotometer in a range of about (300 to 800 nm) of wavelength. The highest UV-visible peak is at 445 nm. The results of color change and UV spectrum were worked in with many recent studies for the biosynthesis of AgNPs from plants extracts[27, 28, 29]. Figure (3) showed the UV spectra recorded after the completion of the reaction.

Figure 3. UV-Vis spectrophotometry of silver nanoparticles by Conocarpus erectus aqueous extract.

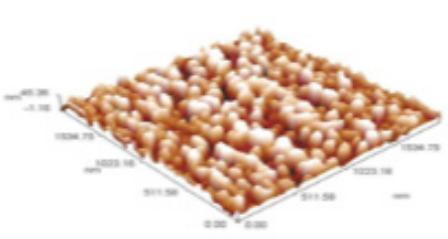


Atomic Force Microscopy (AFM)

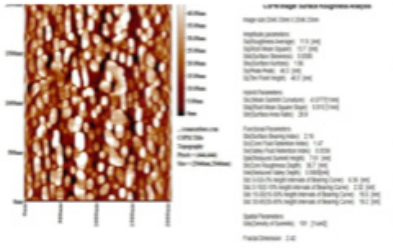
The results which received in this study showed that the biosynthesized AgNPs by *Conocarpous* leaves have average diameter 83.03 nm as shown in Table (3) and Figure (4 a, b and c).

Diameter (nm)<	Volume (%)	Cumulation (%)	Diameter (nm)<	Volume (%)	Cumulation (%)	Diameter (nm)<	Volume (%)	Cumulation (%)
65.00	4.35	4.35	85.00	13.59	60.33			
70.00	14.13	18.48	90.00	10.87	71.20	105.00	5.98	91.85
75.00	11.96	30.43	95.00	7.61	78.80	110.00	8.15	100.00
80.00	16.30	46.74	100.00	7.07	85.87			

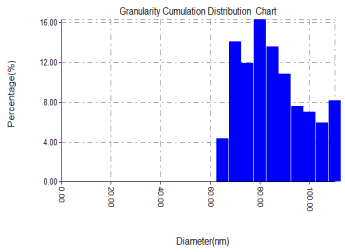
Table 3. The size of sliver Nanoparticles biosynthesis by Conocarpus erectus leaf extract.



(a) 2D image of AgNPs synthesis



(b) 3D image AgNPs synthesis



(c) Granularity distribution chart of AgNPs synthesis.

Figuer 4. Biosynthesized AgNPs by Conocarpus erectus leaf extract under AFM (a, b, c).

Scanning electron microscopy (SEM):

The scanning electron microscope SEM analysis showed the *Conocarpous* leaf plant has great capability to synthesize AgNPs which were predominantly spherical in shape and were uniformly distributed with diameter range (70-78 nm) (Figure 5).

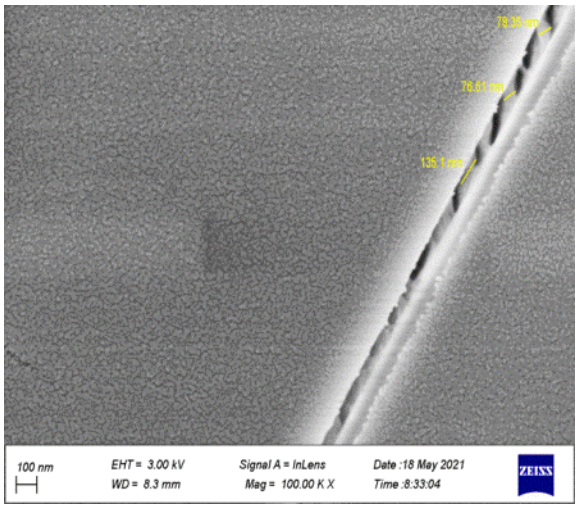


Figure 5. Image of SEM for AgNPs synthesized from *Conocarpus erectus* leaves extract.

Aqueas AgNPs extract

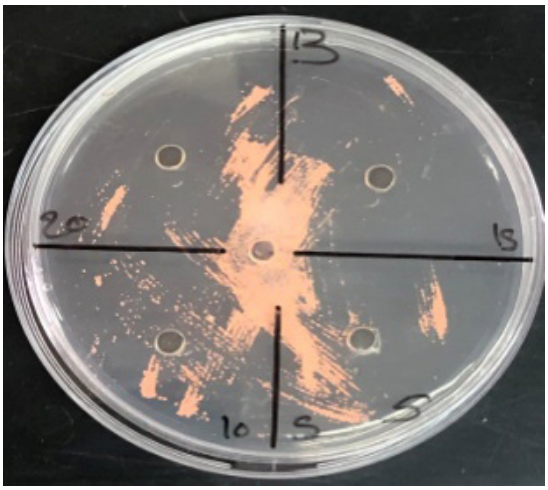
At this part of this research find the result of the antifungal activity of green synthesis AgNPs from *Conocarpous* leaves on five different human pathogenic fungi at different concentrations by sensitivity test.

The result explains the inhibition effect at all fungi cultures, that Cladosporium at lowest concentration 5mg/ml shows highest inhibition zone 19.6 mm with (P≤0.01), while *Rhodotorula-harrison* highest inhibition zone 11.33 mm at concentration 20 mg\ml as at figure (6).

According to table (3) the results were consistent with the result of [30] they reported that AgNPs have antifungal activity so it used to treat fungal infectious diseases. [31]found that antifungal activity of AgNPs was tested against 5 several human pathogens , the maximum inhibitory activity of 20.6 mm was recorded against *Aspergillus fumigatus* followed by *Aspergillus flavus* of (19.3 mm), *Aspergillus niger* of (16.0 mm), *Candidaalbicans* of (13.0 mm) and *Penicillium* (12.3 mm). while [32] showed that The AgNPs prevented the growth of *M. phaseolina, Alternaria alternata*, and *Fusarium oxysporumin* in a dose-dependent manner as compared to the negative control.

Table4.The inhibition zone(mm) of AgNPs of Conocarpous extract effect on fungi were tested in this reaserch.

Fungi	Mean ± SE				LSD value
	Conc . 5 mg\ml	Conc . 10 mg\ml	Conc . 15 mg\ml	Conc . 20 mg\ml	
Cladosporium	19.60 ±0.03	20.00 ±0.00	21.70 ±0.06	24.60 ±0.03	0.377 **
Candida famata	3.67 ±0.07	4.33 ±0.03	5.00 ±0.00	5.00 ±0.06	0.281 NS
Cryptococcus neoformans	3.67 ±0.03	4.33 ±0.07	6.00 ±0.00	6.67 ±0.14	0.302 NS
Milerozyma farinosa	1.33 ±0.03	2.67 ±0.06	6.00 ±0.10	7.00 ±0.15	0.452 **
Rhodotorula harrison	7.33 ±0.18	10.33 ±0.17	11.00 ±0.28	11.33 ±0.32	0.387 **
Candida albicanas	18.30 ±0.17	18.70 ±0.26	20.00 ±0.29	20.00 ±0.06	0.266 NS
LSD value	0.452 **	0.483 **	0.461 **	0.393 **	---
** (P≤0.01).					



Rhodotorula Harrison

Figure 6. Sensitvity test of AgNPs of C. leaves extract on some pathogenic fungi (*Rhodotorula Harrison*).properties.

CONCLUSIONS

The pathogenic fungi were increasing the resistance against various marketed drugs, which is a big problem and increases the need for the discovery of a new antifungal. The present study was aimed to investigate the antifungal potential of a plant leaf *Conocarpus* with three extracts types crud, phenolic, and AgNPs extract. so that the secondary metabolic compounds of *C. erectus* could inhibit fungal growth. Found that phenolic extract has a higher inhibition zone than crud than AgNPs extract.

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