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Use of Green Nano-Extracts in the Control of *Trichophyton Rubrum*

Ghaith Reeda[1],Ban Taha Mohammed[2], Abbas matrood bashi[3]
07706030050Bantmh@gmail.comabbas.matrood@uokerbala.edu.iq
Department of Biology / college of Education for pure Science / Kerbala University / Iraq.[1,2]
Departement of Anastasi and Intensive care /Altuff college /Iraq[3]

Abstract:

The study was conducted in the Postgraduate Laboratory in the College of Education for Pure Sciences and in the Postgraduate Laboratory at the College of Applied Medical Sciences at the University of Karbala and in the Center for Manuscript Preservation , Restoration and Care of Researchers at the Imam Hussain Holy Shrine in Kerbala.The study lasted from 10/10/2019 to 15/10/2020 with the aim of converting the alcoholic extract of *Conocarpus erectus*registered in the Global Genebank that accession numbers MT444957 in previous study , to a hybrid nano silver compound (AgcaNps) and its effect on some phenotypic and microbiological characteristics of *Trichophyton rubrum* IQT-No.1 at different concentrations with different concentrations of the antifungalfluconazole.

Some tests were performed for the hybrid nanoparticles, which is a Spectrophotometer, Fourier transform infrared (FT-IR), X-ray diffraction (XRD) and a Scanning Electron Microscope (SEM) to verify the nanoparticles from the alcoholic plant extract.

Different concentrations (10, 20, 30, 40, 50, 60, 70 and 80mg) were prepared from the alcoholic extract, the(AgcaNps) hybrid nanoparticles, the fluconazole antifungal and the binary between them .

The virulence of the *Trichophyton rubrum*IQT-No.1was tested by its effectiveness on the production of enzymes Protease, Lipase and Keratinase,during 3, 6 and 9 days incubation periods at 25 ° Cwhich showed an enzyme activity directly proportional to the incubation period through the average colony diameter (mm). The enzymes differed in their ability to analyze the culture medium through the diameter of the halo formed around the fungal colony in the case of protease and Keratinase enzymes, and the appearance of the white precipitate in the case of the production of the Lipase enzyme.The results showed that the hybrid nanoparticles (AgcaNps) inhibited the diameter of *Trichophyton rubrum* (8.0) mm more than the alcohol extract of *Conocarpus erectus*. It did not differ significantly from the antifungal fluconazole with the same concentration of 50%. Concerning the dry weight of the fungal colonies, the hybrid nanocomposite AgcaNps was the most inhibiting of *Trichophyton rubrum* with a colony weight of 0.01 mg at a concentration of 40% and it did not differ significantly from the antifungal fluconazole with the same concentration,whereas, the alcoholic extract of *Conocarpus erectus* was the least inhibitor with colony weight 0.02 mg at 40% concentration. The results showed the superiority of the green alcohol extracts of silver nanoparticles in inhibiting the growth of the *Trichophyton rubrum*. The use of the mixture between the hybrid green nano extracts and the antifungal resulted in reducing the dose of the anti-fungal fluconazole are used, because of its negative effects on human cells and all Eukaryotic organisms.

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Many microscopic examinations were performed on the studied fungi and showed the effect of different treatments on the fungal. This effect varied between decomposition or deformations in the fungal mycelium, the separation of protoplasm in some areas, their assembly in other areas in addition to the disappearance of microconidia. The deformation of macroconidia showed internal abnormalities that can be observed, separation of the plasma membrane, and there is another effect that could be observed in the formation of Chlamydospores.

This study was the first of its kind in the field of extracting active compounds and converting them into nanoparticle extracts in inhibiting the growth and efficacy of dermatophytes and reducing the recommended dose of the antifungal used in the case of confusion between them.

KEY WORDS: Dermatophytes, Morphological features, *Trichophyton rubrum* .Nano-silver , Fluconazole antifungal. Green nanoparticles ,*Conocarpus erectus*.

Introduction

Dermatophytes are the most common pathogenic agents of superficial mycoses in humans and animals[1].The epidemiology and distribution ofthesefungal diseases, with the lack of public programmers to prevent or control of these types of infection[2].The *Trichophyton rubrum* was isolated from the types of patients who visited Al-Hassini Teaching Hospital in Karbala Governorate, Iraq, and it was found that this fungus has a high ability to produce the proteolytic enzyme of the protease enzyme in the surface layer of the skin, causing ringworm[3].

The genera *Trichophyton*, *Microsporum* and *Epidermophyton* are among the most pathogenic fungi of the skin and their additives, and the common entrance between these forms is from the pet fungi of keratin present in the layers of the skin keratinophily, and the genus *Trichophyton* is the most complex, as it includes more than 15 speciesit has been classified, as well as many strains of *T. mentagrophytes*[4].Several antifungals have been used as treatment agents for dermatophytes infections, such as ketoconazole, itraconazole,fluconazole, amphotericin B, nystatin, griseofulvin, terbinafine and primycin[5]. In spite of the importanceof antifungals and their ability to eliminate these organisms, they are side effects, as well as the emergence of new strains of the fungal races that can increase their ability to resistant and become completely resistant over time[6].Therefore , there are many studies focused on the use of medical plants to extract some alcoholic extracts of *Conocarpus erectus, Eucalyptus globulus, Salvia rosmarinus, Thymus vulgaris and Zingibar officinale*a phenols [7]. The use of *Marasmius plamivorus*filtratehad a significant effect on the determent growth and reproduction of pathogenic fungi *T. rubrum* ,where there was a significant reduction in the regulation of gene expression for Citrate Synthase and Serine Protase compared to the gene calibration result of the control group equivalent to an expression[3].The use of nano zinc has more effective effect than mineral zinc to decrease the growth of *T.rubrum* and *Microsporum canise* and has an important role in reducing the amount of antifungal used[8].Given the great importance of this fungus and its great impact on human health and to find an optimal treatment for it without a side effects and high cost of traditional drugs, this study aimed to convert alcoholic plant extracts of *Conocarpus erectus* into green nanoparticles , study their efficiency and effectiveness in control the *T. rubrum* and its comparison with some anti-fungal drugs (Fluconazole) and alcoholic plant extracts of the same plant.

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MATERIALS AND METHODS

Trichophyton rubrum isolate IQT-No.1 was obtained from Prof. Ban Taha Mohammad from the postgraduate laboratory at the College of Education for Pure Sciences at the University of Karbala, diagnosed and registered in the Global Genbank under the serial number (MK167434.1)[3].The isolate was activated according to what was mentioned in [9] then it was planted on SDA medium, and its phenotypic and microscopic properties were studied before and after the subsequent treatments. The enzymatic activity of the fungus keratinase, protease and lipase was measured to verify the virulence of the fungal isolate.

The leaves of the *Conocarpus erectus*, were obtained from Prof. Dr. Ban Taha Muhammad from the Postgraduate Laboratory at the College of Education for Pure Sciences at the University of Karbala, which was diagnosed and classified in the Genbank, which carries the sequence MT444957 accession number[7].

The method used by Hernandezi *et, al*,(1994) [10]was followed in preparing the alcoholic extract, whereby (50) grams of dry leaves powder were taken , mixed with (500) mL of ethyl alcohol (70%) , then placed in a vibrating incubator for a period of two hour , left for (24) hours at room temperature, after which the mixture is filtered using several layers of medical gauze , then the extract is filtered using 0.1 Whatman No 1. To obtain a clear solution, dry the extract using the oven at 40 ° C, and store in the refrigerator until used.

Reduction of silver nitrate(AgNO3) with *Conocarpus erectus*dry extract leaves :

Twograms of dried plant extract were added to 2 ml of silver nitrate solution (prepared from dissolving 0.168 g which equals to 0.01 mol) in 100 ml of deionized water, then put on a magnetic stirrer device at a temperature of 40^{o}C. When the color change of the aqueous solution was observed from yellow to red-brown, the permeability of the solution was measured in the UV Visible Spectrophotometer, where the reading was between 190 -500 nm, and the beam began to rise at 300 nm and reached the highest peak at 400 nm. At 500 nanometers, the beam falls, indicating the spectrum of nanoscale silver and silver reduction.The solution was placed in the shaker incubator for 24 hours, then the precipitate was separated by a centrifuge, then washed with deionized water several times and then dried at a temperature of 50 ^{O} C, then grinded well to obtain a fine powder, and stored in the refrigerator until use in subsequent experiments. Also, upon use, the obtained powder is dissolved with DMSO (Dimethyl sulfoxide), as it either dissolves 95-99% of the substance or works to disperse the substance and turn it into a semi-soluble, and then both alcohol and aqueous dilutions were done.[11].

Agricultural media used:

Sabouraud's dextrose agar medium: sixty fife grams of medium powder were dissolved in amount of distilled water and the volume was comleted to 1 liter according to the manufacturer's instructions (HIMEDIA), poured into petri dishes according to the purpose of the experiment.

Potato dextrose agar (PDA): According to the manufacturer's instructionsit wasprepared by dissolving 39g of the medium powder in 1L of distilled water.

Skimmed - milk Agar: Dissolving of 5 g of skimmed milk in 50 ml of distilled water, 10 g of agar were dissolved in 450 ml of distilled water in another flask, the pH was adjusted to 7, the

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two solutions were separated, then cooled to 45 ° C and then mixed together. The medium was used to reveal the ability of the fungi to produce the protease enzyme by the emergence of a transparent halo around the fungal colonies**.**

Tween 80 Agar: The media was prepared as the following materials (8 g peptone, 5 g NaCl and 0.1 g $CaCl_2$) in 1L of distilled water and 20 g of agar, 10 ml of Tween 80 was added , the pH was adjusted to 6.8. The medium was used for the purpose of investigating the ability of yeasts and fungi to produce the lipase enzyme by forming a visible white precipitate under the growth or white crystals immersed in the medium around the colony culture media**.**

Keratin agar: Chicken feathers were used as a source of keratin, where a large amount of chicken was collected, cut to a size of 1 cm, washed with chloroform and methanol alcohol, with a volume ratio of 1: 1, and then washed with distilled water , dried in the sun [12]. The feathers were placed in 10 ml of a solution of dimethyl sulfoxide (DMSO) for 24 hours, and then 10 ml of acetone was added and left for 30 minutes. Then it was filtered with filter paper and the filtrate was taken and added to the medium of the mineral salt pellets containing K_2HPO_4 1.5 g / liter. ; $MgSO_4.7H_2O$ 0.05 g / l; $CaCl_2$ 0.025 g / l; $FeSO_4.7H_2O$ 0.015 g / l; $ZnSO_4.7H_2O$ 0.005 g / l; 2 agar, 100 ml distilled water and the pH was ajuested to 7.5, where it was indicated that the ability of fungi to produce keratin enzyme is effective forming aclear halo zone around the fungal colony .

To all of the above media, 250 mg/l of Chloramphenicol antibiotic was added before sterilization, and then sterilized by autoclave at 121 ^{O}C under 1.5 pressure for 20 min.

The methods of Khanzada *et al*.,(2006) [13]were used**.** The plant alcoholic extracts and the hybrid nanoparticles (AgcNps) were mixed each of the culture media SDA before solidification, taking into account 10 mg of the extracts. It was dissolved in 5 mg of distilled water and added to 95 ml of the culture medium and poured into 6 plates at eight concentrations 10,20,30,40,50,60,70 and 80mg encoding three replicates for each concentration, in addition to the control.After the solidification of media, a hole was made in the center of each plate with a cork borer with a diameter of 7 mm. Then the plates were inoculated with the same diameter of fungal inoculum. All plates were incubated at 25 ° C for two weeks. The diameters of the developing colony were measured (average of two perpendicular diameters), and the results were recorded.

Phenotype,and microscopicfeatures were studied . Glass slides were prepared and the fungi were examined under a compound microscope with a magnification power of 40 X after staining them with the lactophenole cotton blue to study the effect of the treatments on vegetative and reproductive growth[14].

For the purpose of testing the effect of the extracts on the dry weight of fungi, the plant extract of *Conocarpus erectus*, the antifungal fluconazole and the hybrid nanocomposite (AgcNps) were mixed separately with the sterile SDB culture medium , eight concentrations (10,20,30,40,50. 60,70,80) mg, at a rate of three replicates for each concentration, in addition to the control.The used of plastic tubes of 100 ml in each of 20 ml of culture medium, incubation at 25 ^{0} C for two weeks, the dry weight was matured by using a sensitive electrical balance .

The effect of the prepared nanoparticles on the growth of *Trichophyton rubrum* was evaluated on the basis of different combinations:The alcohol extract of *Conocarpus* was synthesized with the anti-fungal fluconazole, with the concentration inhibiting the studied fungi and in the following proportions 25% .50% .75%. The same method was used by the synthesis of the hybrid nanoparticles (AgcNps) with the antifungal fluconazole.

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Statistical analysis: Factorial experiment within Completely Randomized Design (CRD) was adopted. The means of tretments were compared using the Least Significant Difference (LSD) at a probability level of 0.05 .Genstat software was used in the statistical analysis.

Results and dictation :

Phenotypic and microscopic properties of *Trichophyton rubrum* IQT-No.1:, *Trichophyton rubrum* showed in Figure 1 , white, flat and slightly elevated SDA colonies, with a soft cottony to velvety fluffy loose. The back side of the colony showed a brownish-yellow to reddish-brown color. The growth rate of the fungal culture was 8 cm after 14 days of incubation at 25 ° C. The microscopic examination showed an abundance of production of microconidia, spherical to pear-shaped, and in a spiral arrangement along the mycelium, and macroconidia with a cylindrical shape resembling a cigar shape with thick, smooth multicellular walls between 6-8 cells.These features are the same as that listed in [15]

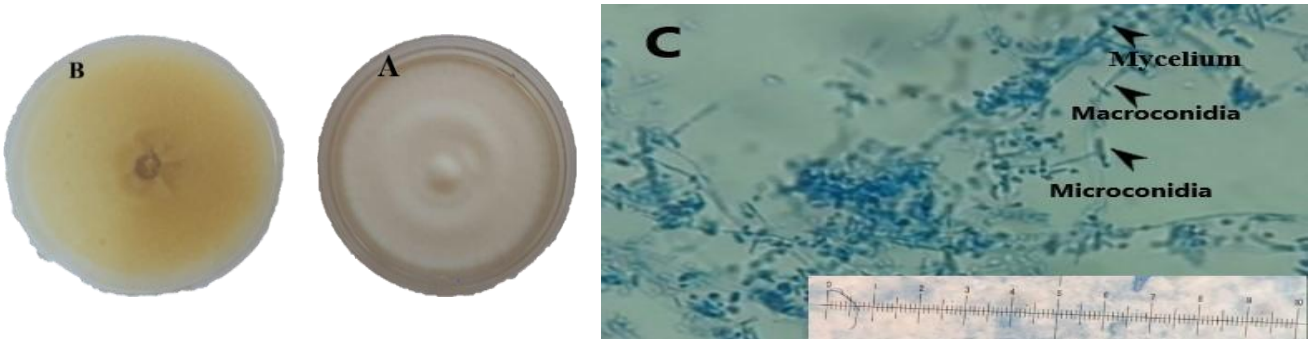

Figure 1: Phenotypic and microscopic features of *Trichophyton rubrum* On SDA medium at a temperature of 25 ^{0} C at a age of 14 days.
A = The upper surface of the colony of Trichophyton rubrum
B = Posterior faceof the colony of Trichophyton rubrum
C = the microscopic form of the fungus *Trichophyton rubrum*, in which the mycelium, Macroconidia and Microconidia appear after with lactophenol cotton blue with 40X magnification.

three enzymes, namely Keratinase, Lipase and Protease, in culture media suitable for each enzyme. The amount of enzyme produced is proportional to the average of the colony diameter (mm) around the colony fungal in the case of the enzymes Keratinase and Protease, or in the form of a white precipitate around the colony in the case of Lipase as shown in the figure 2.These virulence are as the same results that obtained from Yassin and Mohammed ,2021 [16].

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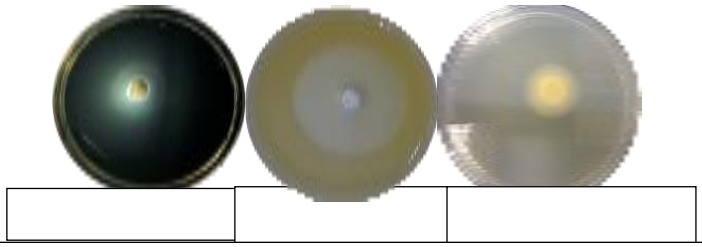

Figure 2: Enzyme production by Trichophyton rubrum on the appropriate culture media for each enzyme at a temperature of 25 0 C and an age of 9 days.
Keratinase = Keratinase production from on the medium of keratin agar
Lipase = Lipase production from on Tween 80 Agar medium
Protase = Protase production from on medium skimmed milk agar.

Figure 3, shows that the highest productivity of enzymes was during the incubation period of 9 days. The protease enzyme gave a high productivity during the exhibition period of 57.00 mm at LSD $_{0.05}$' followed by the keratinase enzyme with a halo diameter of 36.00 mm, while the lipase enzyme had a minimum diameter of the fungal colony of 39.17 mm.This result is similar to what has been reported by [16] on the same fungus .A study presented by Al-Masoudi and his group (2020) showed the ability of *Trichophyton rubrum* to produce the protease enzyme and increased gene expression of this enzyme when treated with *Marasmius palmyphorous*[17].Also, the *Trichophyton* has the ability to produce the maximum keratinase at a temperature of 27^{o}C after four days inocubation in the medium contains chicken feathers powder at pH= 8.5[12].It was also noticed that the virulence of the fungus is due to its power production from hemolytic enzymes and epidermal cells, and its lipase repellents.[18].The results of this study agreed with the study of Kadhim and his groups (2015)[19], which found that the highest period of production of the protease enzyme was after 9 days. Also, these results were in agreement with [20]where they showed that the best incubation period for the production of the enzyme is 9 days.

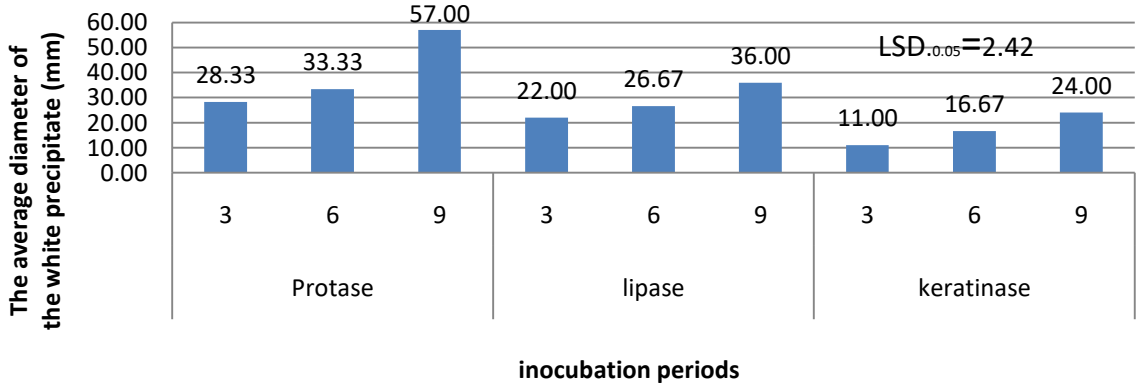

Figure 3: A clear halo zone (mm) resulting from the activity of the enzymes, protease and keratinase , and white crystals immersed from the3,6,9 days of incubation at 28^{0}C.

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Characteristics of nanoparticls prepared by FTIR, SEM, XRD: Figure 4, indicates the spectrum analysis in blue color (representing the remains of the conocarpus), while the red color represents the reduced silver in the form of nanocrystalline silver and its peaks at 42 angles equal to 14.73 and at an angle of 21.93 is very clear, as well as at an angle of 35.92 these peaks appear in red It belongs to nanoscale silver, and the evidence that conocarpus has reduced nanocrystals is the appearance of these picas at the mentioned angles.

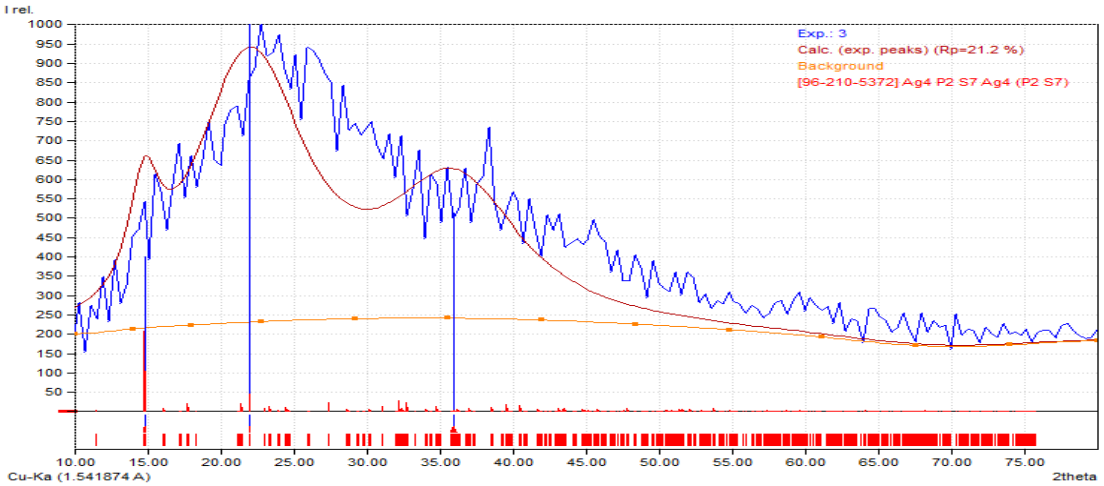

Figure 4: Spectrum analysis of the preparation of the nanocomposite silver nitrate from the alcoholic extract of *Conocarpus erectus*with silver nitrate.

Silver nanoparticles are shown in the shape of a cauliflower in Fig. 4Picture 1.Also, in Image 2, was enlarged and the distance became 5 microns, so the nanoparticles silver appears in the shape of a cauliflower, and this composition is very clear. In Image 3, the image was enlarged and the distance became 5 microns, so the nanoparticles silver appears in the shape of a cauliflower, and this composition is very clear. The Images 4-7 have been enlarged by a distance of 2 microns and can see the silver nanoparticles ,their clusters and their measurements can be clearly measured, which indicates that they are nanoparticles .The images 5-7 are enlarged, and the silver nanoparticles in the shape of a cauliflower were seen, and clusters were clear as the image is enlarged. in picture 8 the clear clusters are shown in them and see grains indicating that they are silver nanoparticles, In Picture 9,clearly shows the particles, Picture 10 shows the alcoholic *Conocarpus* with silver extract, and the silver that appears has been visibly enlarged. and Picture 11, analysis of specific locations , In the First, the nanoparticle for silver and determined between the yellow-colored lines, which represent the diameter of the nanoparticle, where the results appeared 18.88 nanoparticles , the second place (a second measurement was taken) and the molecule was placed between two lines, where the results showed 19.91 nano and in the third place, one molecule from the group was taken, counted, and measured, where the results appeared to be equal to 29.59 nanoparticles, indicating that it was worked in the nanoscale field and that this silver is nanoscale and reduced by *Conocarpus.*

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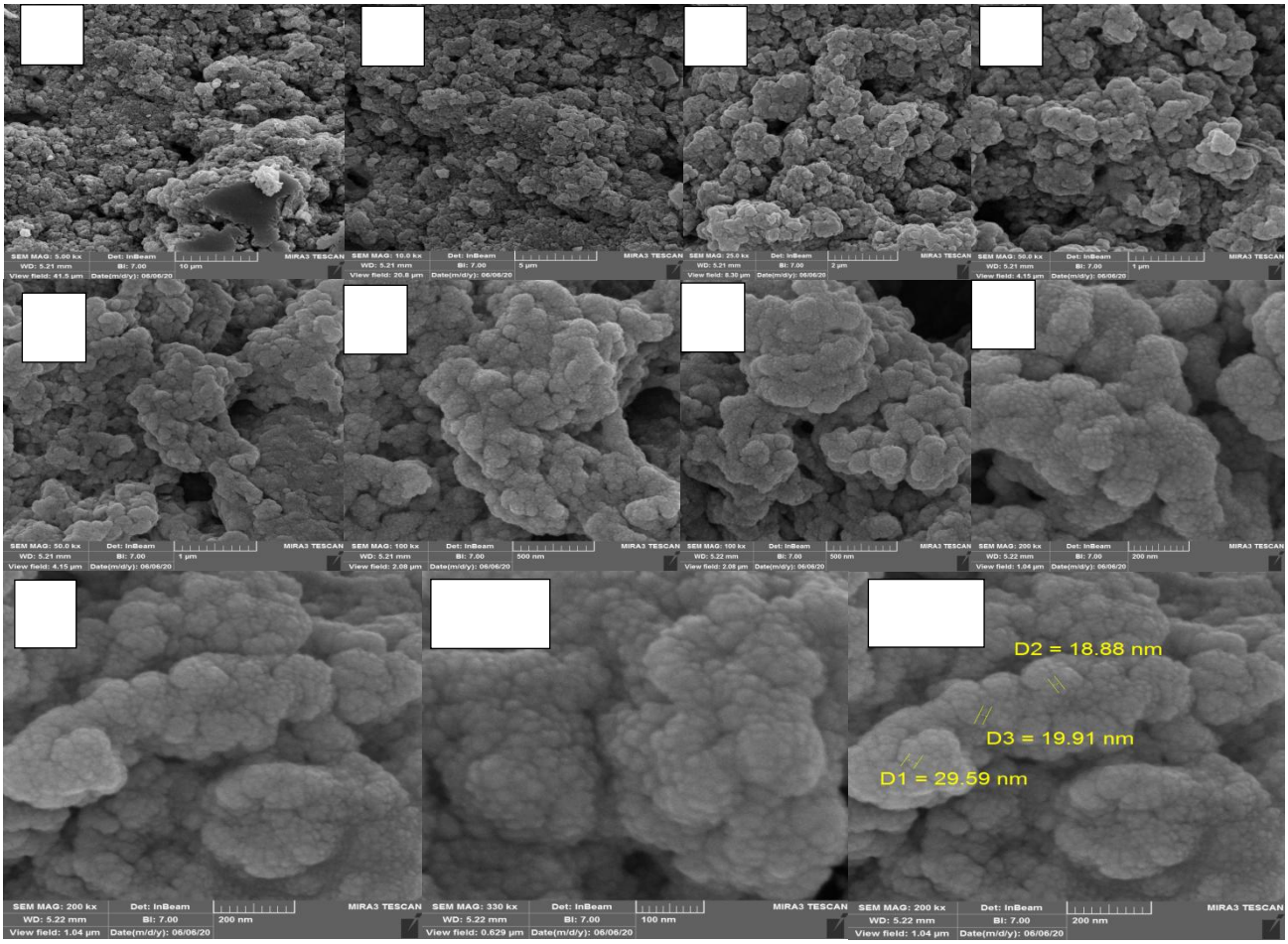

Figure 4: SEM analysis of the nanocomposite preparation of silver nitrate from the alcoholic extract of *Conocarpus*with silver nitrate. Pictures 1-11 indicate different enlargement power.

In figure 5, group 3417 is shown, indicating the OH group, while bundles 2926 and 2854 are indicative of the aqueous group as it has shifted somewhat from its place in the aqueous state.

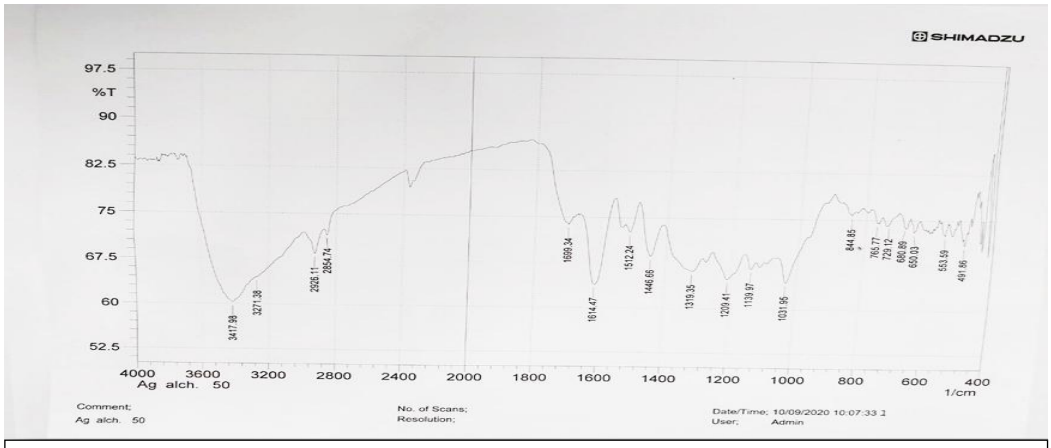

Figure 4: FTIR analysis of the nanoparticles preparation of silver nitrate from the alcoholic extract of *Conocarpus* with silver nitrate

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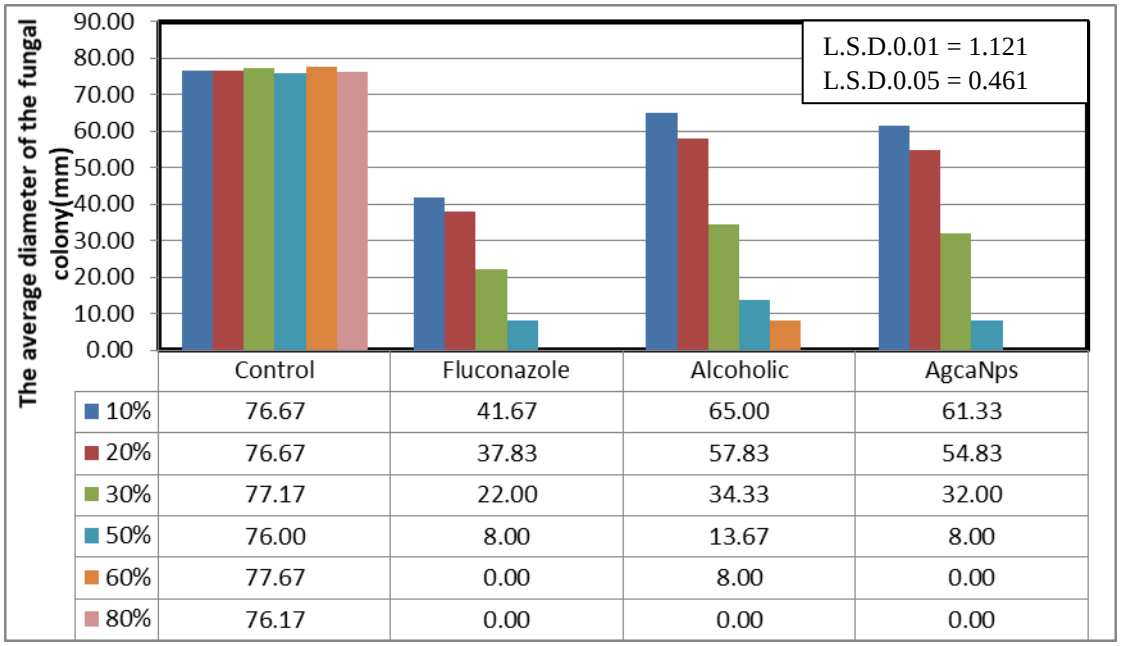
Bundles 1614 and 1629 denote stretchiness extending to the OH bond present in alcohols and also present in the extract, while bundles 1645, 1319, and 1219 all belong to the group of amines. All these analysis referd to excellent nanoparticles produced within the range of silver nanoparticls [21]. The use of silver nanoparticles in various aspects of life such as energy production, biomarking and antimicrobial agents ,the green nanoparticls industry process is based on chemical and physical methods and its benefits are: environmentally friendly, cost effective and can be easily expanded in large-scale nanoparticle industry, moreover there is no need to use high temperature, pressure, energy and toxic chemicals[22] .

Evaluate the prepared nanocomposites in the growth of *Trichophyton rubrum*: The results shown in Figure (5) revealed the interaction between the types of extracts and the concentrations decreased in the diameter of the fungal colonies with increasing the concentration of the hybrid nanoparticles extract for both the silver and the alcoholic extract, as well as with the antifungal fluconazole. Silver hybrid nanoparticles were not significantly different from the antifungal Fluconazole, which in turn differed from the control. The fungus also showed significant differences between it and the concentrations of all extracts at the levels 0.05 and 0.01.



	Control	Fluconazole	Alcoholic	AgcaNps
10%	76.67	41.67	65.00	61.33
20%	76.67	37.83	57.83	54.83
30%	77.17	22.00	34.33	32.00
50%	76.00	8.00	13.67	8.00
60%	77.67	0.00	8.00	0.00
80%	76.17	0.00	0.00	0.00

Figure 5: Interaction between extract types and concentration in the mean fungal colony diameter of *Trichophyton rubrum* (mm) growing on SDA medium at 2 ± 25 0 C for 14 days

It is evident from Fig. 6, that there is a decrease in the mean weight of the fungal colonies with an increase in the concentration of extracts for all extracts and for the fungi under study compared to the control. Note that the antifungal was the most effective on the dry weight of the fungi with no signfecant with the AgcaNps.In this study, the alcoholic extract of the *Conocarpus*

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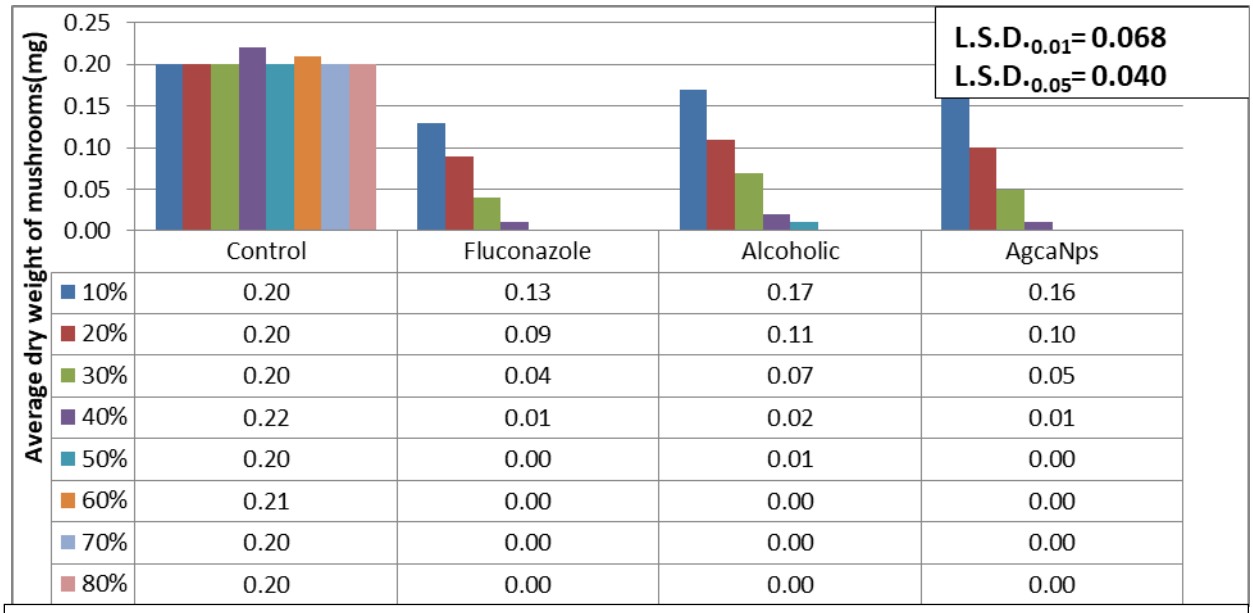
was used, as the results of the effectiveness of the alcoholic extracts in inhibiting the *Trichophyton rubrum*which were in agreement with [23].



	Control	Fluconazole	Alcoholic	AgcaNps
10%	0.20	0.13	0.17	0.16
20%	0.20	0.09	0.11	0.10
30%	0.20	0.04	0.07	0.05
40%	0.22	0.01	0.02	0.01
50%	0.20	0.00	0.01	0.00
60%	0.21	0.00	0.00	0.00
70%	0.20	0.00	0.00	0.00
80%	0.20	0.00	0.00	0.00

Figure 6: Interaction between extract types and concentration in the average dry weight fungal colony of *Trichophyton rubrum* (mg) growing on SDB medium at 2 ± 25 ^{0} C for 14 days.

The present study appeared that a hybrid nanoparticls resulting from silver reduction from silver nitrate was used with alcoholic extract of Conocarpus because silver ions Ag and silver-Ag compounds had strong antimicrobial effects[24]. These inorganic nanoparticls have a clear advantage over conventional chemicals and antimicrobial agents,the most important problem caused by microbes was their multiple resistance to treatments,so , finding an alternative way to beat the drug it would of importance . Resistance to various microorganisms in medical devices is urgently needed. Therefore, Ag ions and Ag salts have been used as antimicrobial agents in various fields due to their ability to inhibit growth against microorganisms [25].

Phenotypic and microscopic properties of *Trichophyton rubrum* IQM-No.1 after the tretments :

Figure 7, shows the effect of the antifungal fluconazole on the fungus. From the phenotypic, the fungal colonies appear to be receding and tend to rise towards the surface, while from the back side of the colonies there is a concentration in the yellow pigment compared to the control (figure 1) . In the same figure 7, it is seen in the microscopic field of the fungus, with the discress the number of the microconidia and Macroconidia. The fungal hypha was devoid of protoplasmic substance , with separation of the protoplasmic membrane (c). Also, fungal filaments breakdown and decomposition can be observed (e).

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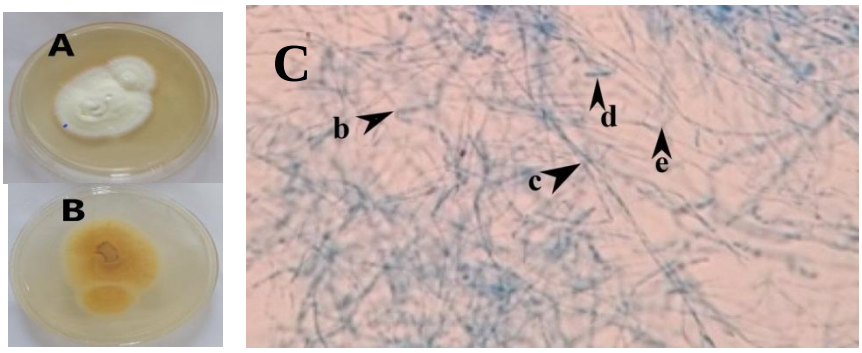

Figure 7: Effect of Fluconazole on *Trichophyton rubrum* on SDA medium at 25 0 C and 14 days old.
A = The upper surface of the colony of *Trichophyton rubrum*
B = Posterior face of the colony of *Trichophyton rubrum*
C = The microscopic form of *Trichophyton rubrum* after staining with lactophenol cottone bluein 40X magnification
b = aggregation of protoplasm with detachment of plasma membrane, c = mycoplasmic hypha devoid of protoplasmic contents, d = Macroconidia, e = mycelium lysis

Figure 8, appears when treating with alcoholic extract of *Conocarpus* leaves, from the phenotypic side the fungal colonies appear to be receding and tend to rise towards the surface, while from the back side of the colonies there is a concentration in the yellow pigment compared to the control (figure 1). In the same figure 8, it is seen in the microscopic field of the fungus with the disappearance of the Macro and microconidia , and grouping of protoplasm indicated by (b) is shown in the figure. With detachment of the protoplasmic membrane.

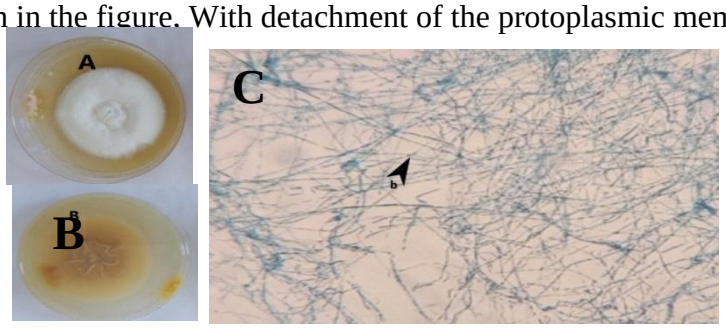

Figure8: Effect of alcoholic extract of Quinocarpus leaves on Trichophyton rubrum and Microsporum canis on SDA medium at 25 0 C at 14 days age.
A = The upper surface of the colony of Trichophyton rubrum
B = Posterior face of the colony of Trichophyton rubrum
C = The microscopic shape of Trichophyton rubrum after staining its mycelium form with the blue lactophenol dye with a force of 40X magnification.
b = assembly of protoplasm with plasma membrane detachment

Figure 9, shows when treating with a hybrid alcoholic extract with silver nanoparticles (AgcaNps), the fungal colonies appear colored in a yellowish-brown color phenotype, and there are no significant phenotypic differences compared to the control. However, the microscopic examination showed the differences. Protoplasmosis on its way to degradation, with the presence of chlamydospore.

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Table 2: The effect of different combinations of extracts with the antifungal Fluconazol on *Trichophyton rubrum* (m[illegible] diameter rate on SDA medium at 25 ^{0} C at 14 days age.

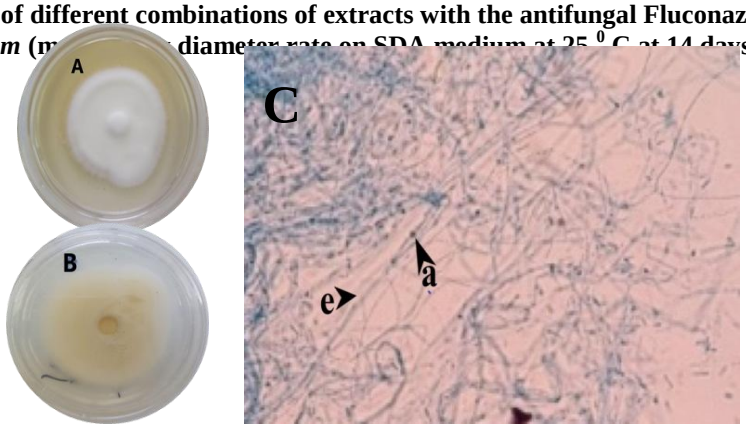

Figure 9: Effect of a hybrid alcoholic extract with silver nanoparticls on *Trichophyton rubrum* on SDA medium at 25 0 C at 14 days age.
A = The upper surface of the colony of Trichophyton rubrum
B = Posterior face of the colony of Trichophyton rubrum
C = the microscopic form of Trichophyton rubrum after staining fungi with lactophenol cotton blue in 40X
a = chlamydospore, e = lysis of mycelium

Giving evidence of fungal poisoning with different treatments, which gave results in the direction of damage to the fungus, and the results of the damage were similar to what was mentioned in despite the different treatments and their concentrations[25][20][28]

Evaluate the different combinations of the different extracts and the antifungal fluconazole in the growth of *Trichophyton rubrum*:The results indicated in Table 2, that using the combination of alcoholic extract or AgcaNps with the antifungal fluconazole (50% and 75%) led to a greater inhibition of *Trichophyton* than using alcoholic extract or (AgcaNps) separately. With the same concentration, as the treatment containing AgcaNps + (fluconazole) with a synthesis rate of 75% gave the least diameter of the fungal colony, followed by the treatment (AgcaNps + fluconazole) with a combination of 50% at the 0.01 significant level.The production of hybrid nanoparticls is one of the modern environmentally friendly methods [11]. Therefore, the conversion of plant extracts into a nanocomposite with silver nitrate showed its effectiveness against fungi [29].The results showed the superiority of alcoholic extract with silver nitrification in Inhibition of *T.rubrum*.

Conclusions: The use of mixing green hybrid nanoparticls extracts led to a decrease in the dose of the antifungal used fluconazole, due to its negative effects on human cells and all eukaryotes. This study is considered the first of its kind in the field of extracting effective compounds and converting them into nanoparticls extracts in inhibiting the growth and efficacy of dermatophytes and reducing the recommended dose of the antifungal used in the case of confusion between them.

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*Fluconazol(mg)+Extracts(mg)	Cmbination %	Colony diameter (mm)
control	control	77.00
fluconazole(15mg) + alcoholic(45mg)	25%	13.33
fluconazole (30mg)+ alcoholic (30 mg)	50%	12.33
fluconazole (45mg)+ alcoholic(15mg)	75%	11.00
(0.0mg)fluconazole+ alcoholic (60mg)	0.0%	13.33
fluconazole(12.5 mg)+ AgcaNps (37.5 mg)	25%	15.00
fluconazole(25 mg) + AgcaNps (25mg)	50%	13.67
fluconazole(37.5 mg)+ AgcaNps (12.5mg)	75%	10.67
fluconazole (0.0mg) + AgcaNps (50mg)	0.0%	17.00

$LSD_{0.01}$=0.93
*=three replications for each treatment.

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