WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 7, 1345-1353. Research Article ISSN 2277– 7105

SYNTHESIS OF GOLD NANOPARTICLES FROM NATURAL HONEY AND ITS APPLICATION IN ANTIFUNGAL ACTIVITY

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Article Received on
10 Feb. 2018,

Revised on 02 Mar. 2018,
Accepted on 21 Mar. 2018,

DOI: 10.20959/wjpr20187-11721

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ABSTRACT

The fungal species are major human pathogenic fungi. It's affect mucosal membrane, tissue damage and contaminate food. *Aspergillus* species are mostly contaminating food materials. This article main aim to control the growth of *candida* species by using gold nanoparticles. Because *Candida* affect kid in 13yrs child, infect women vaginal region and commonly affect oral infection. *Candida* affect the humans has malnutrient and interfere. The *Candida* species has ability to drug resistant for both antimicrobial and antifungal drugs. For that using natural honey to synthesis gold nanoparticle is biological method. These nanoparticles are eco-friendly to environment and non-harmful to humans. The nanoparticle characterized by using UV-spectrum, TEM, EDX, FTIR. Performing the minimum inhibitory concentration (MIC) well cutting method different volume of gold nanoparticle control the growth of candida species and Aspergillus.

KEYWORDS: Natural honey, gold nanoparticle, antifungal activity, MIC.

INTRODUCTION

Candida is an opportunistic fungal pathogen, they are present on surface of gastrointestinal and genitourinary tract, skin of human, mucosal oral cavity, vagina, oesophageal and various clinical mucocutaneous growth of blood stream.[1-2] In medical team affect the women vaginal is Vulvovaginal Candidiasis(VVC), repeated same vaginal infection is Recurrent Vulvovaginal Candidiasis(VVC), oral infection is Oral Pharyngeal Candidiasis(OPC). This OPC affect only common HIV patient.[3] More than 200 species are available in *Candida* but

some species only able to cause diseases and infection to human begin. *Candida* able to spread all major organ and from colonies.

In that 90% of invasive infection caused by Candida species like *C. albicans, C. glabrate, C. parapsilosis, C. tropicalis and C. krusei. C. glabrate* present in the blood stream.[4-5] *C.guilliermomdii, C. kefyr, C. rugosa, C. dubliniensis and C. famata* are world-wide pathogens.[6] Candida species has ability to virulence factors like host defences, adherence, biofilm formation and tissue damaging hydrolytic enzymes such as protease, haemolysis[7] The most candida resident to antimicrobial drugs such as nystatin, amphotericin B, fluconazole, flu cytosine, ketoconazole, Iitraconazole.[8] The *candida* species affect mostly HIV, AIDS patient because leads to malnutrient and interfere with the absorption of mediation.

The main aim to solve problem of drug resident of candida. For that we use gold nanoparticle to treat control the candida growth. The synthesis of gold nanoparticle by using natural honey is biological method. It's non-harmful to environment and human health.[9-10] We use different volume of gold nanoparticle in Muller Hinton agar by the method of minimal inhibitory concentration (MIC).[11] This method shows resident and sensitive of *candida* species in different volumes. Its use to design the drug for *candida* species.

MATERIALS AND METHODS

Pure Honey was collected from Kerala (India), $HAuCl_4.3H_20$ purchase from Mumbai, Collect the fungal culture from PSG hospital in Coimbatore (India), Sabouraud Dextrose Agar (SDA) Hi-media, Muller Hinton agar Hi-media.

Synthesis of AuNP's

The synthesis of gold nanoparticles from natural honey. The honey is diluted to water, 20g of honey is diluted with 70ml of distilled water. Different volume of honey (15, 20, 25, and 30) ml is used for synthesis nanoparticles. The 50mg of HAuCL4 is dissolved in 120ml distilled water. The 10ml of honey is added to 30ml of HAuCL4.The varied concentration of honey is taken as 15(s1), 20(s2), 25(s3), 30(s4). The honey is a reducing agent to give purple color at room temperature within 3hrs.[12-13]

Characterization of Nanoparticle

After synthesis of gold nanoparticles from natural honey. Then the sample was characterized by using UV-Visible spectrum to absorption the peak of gold nanoparticles. The highest peak of (s4) is centrifuged at 10,000rpm for 30min and collect the pellet, analysis the sample in TEM gives the high resolution image and identified size and shape of AuNPs. The energy dispersive X-ray (EDX) at acceleration voltage of 120KV. FTIR is use to identify which biomolecules produce the gold nanoparticles. To detect Capping of nanoparticles and stabilization of gold nanoparticles. TEM, EDX, FTIR analysis the pellet was submitted to PSG Tech, Nanotechnology department, Coimbatore.

Anti-Fungal Activity

Isolate pure culture

Collect the fungal culture from patient from hospital is *Candida* species. The *Candida* species collect from hospital subculture in SDA plate and appearance of culture in SDA shown(fig:5) stored for further work. Isolate *Aspergillus* fungi culture from contaminated or spoiled foods. For the *Aspergillus* collect the spoiled food items, take 1g of food to grain and diluted in 100ml distilled water is 10^{-1}. In that take 1ml serial diluted to 10^{-2} and the same steps are repeated to 10^{-4} dilution. Use 2,3,4 dilutions because fungi isolation use this dilution. Take 0.1ml of sample is spread in SDA plate. For fungi isolation selective media is SDA. Incubate the plates in 28° c for 2-3 days.[16] After done lacto phenol cotton blue identified Aspergillus culture. Then streak on SDA plate for pure culture and the *Aspergillus* appearance in SDA shows (fig:6) stored for further works.

Minimal inhibitory concentration(MIC)

MIC is an agar diffusion method used to identify the highest and lowest concentration to control the growth of fungal culture. It's give the result like zone indicates resistant and sensitive.[17] The resistant is able to tolerant and growth in plates but sensitive is not able to growth nanoparticle inhibit the growth of fungi culture. Prepare muller hinton agar sterilized and poured to Petri dishes. The synthesised gold nanoparticle are centrifuged in 10,000rpm for 10min. collect 1g of pellet, diluted in 100ml distilled water is 10^{-1}(18-19). In that take different volume like 100µl,150 µl,200 µl,250 µl. after solidified spread the fungal culture and cut the well in agar and added the different volume of gold nanoparticle in that well. The plates are incubated at 28°c for 24hr. After incubation observed the zone which concentration

inhibit the growth of fungal culture (13,20-21) (fig:7). Measure the zones gives result in *Candida* and *Aspergillus* shown in (table 1).

RESULT AND DISCUSSION

Spectrum range at 550nm absorbance the peak of nanoparticles. In this study at the concentration of 15ml-169.45, 20ml-244.42, 25ml-287.65, 30ml-327.52 the value drawn as graph (1). The synthesis gold increases in (S4). TEM image to conform the size and morphology of gold nanoparticles. Gold (s4) consist the spherical shape it averages size ± 50nm (Fig. 1). The excess honey to reduce the aqueous HAuCi4 it acting as capping agent shaped spherical nanoparticles. Gold[S4] consists also triangular shape is ±20nm (Fig: 2) reduction of HAuCL4 capping strongly to shape Nano triangular.[12-13] The energy dispersive X-ray (EDX) pattern recorded gold nanoparticles. The analysis of sample (S4) indicates of gold nanoparticles signals confirms the encapsulated matrix of honey. The peaks of carbon, oxygen, detected reducing of honey. FTIR detected the capping and stabilization of AuNPs. The sample (S_4) absorptions intense are 1640, 1040 cm1. The IR band at 1640 has indicates the amide I and II bands of protein are occurring.[14] The band 1040 band presence of C=0 indicates the –COOH group bound on gold Nano particles.[15] FTIR bands are indicates possible compound of protein and –COOH group based on gold Nano particle are synthesised.

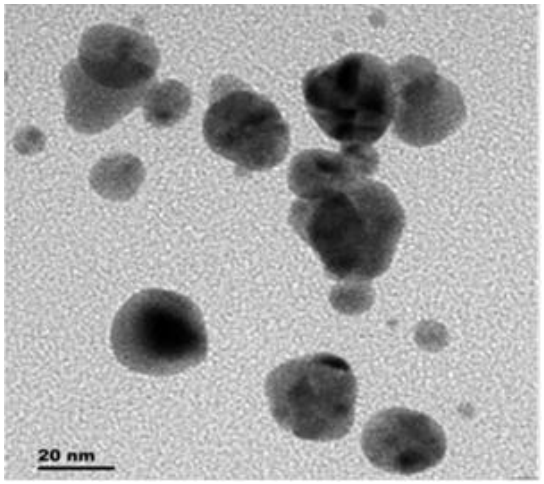


Fig. 1: (s4) AuNPs Spherical Shape

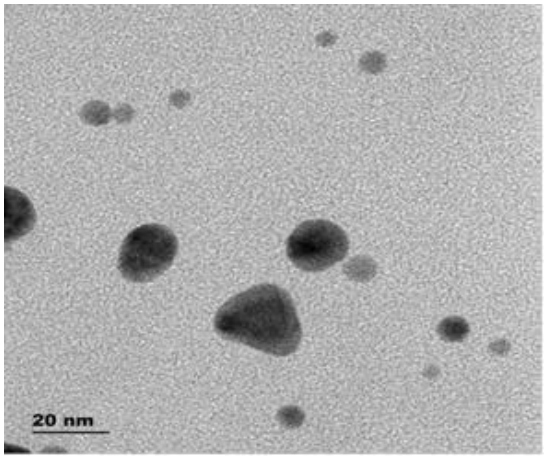
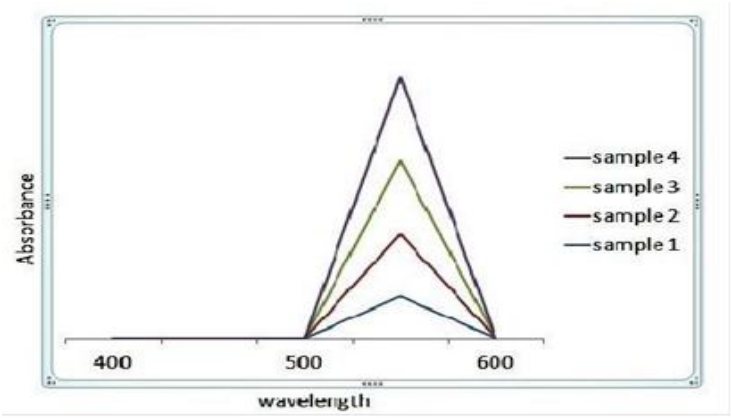


Fig. 2: (s4) AuNPs Triangular Shape.



Gra. 1: UV-Visible Spectrum.

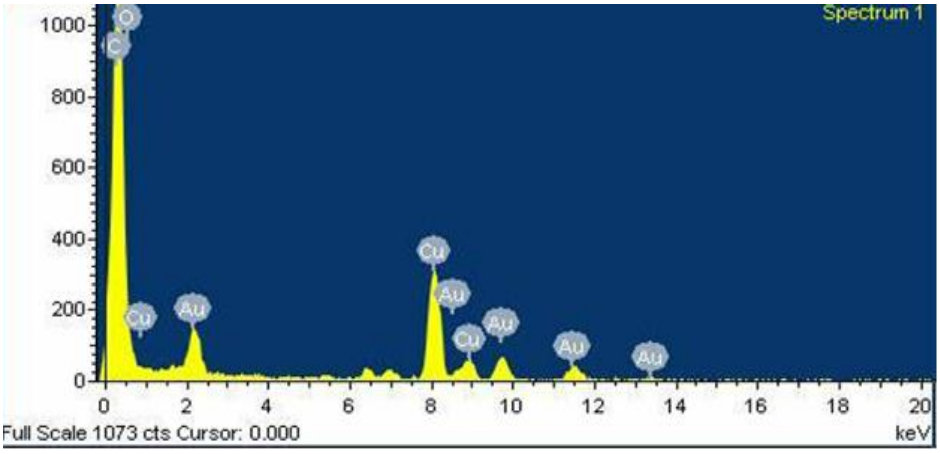


Fig. 3: (s4) AuNPs Matrix of honey.

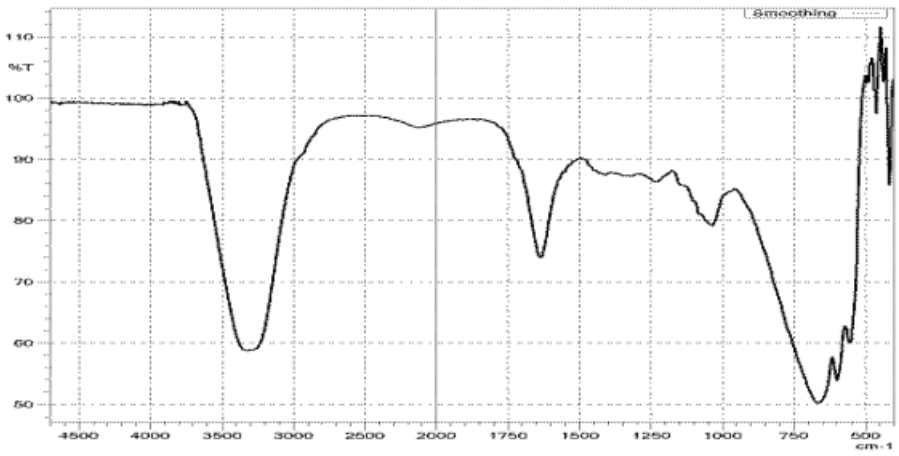


Fig. 4: (S_4) FTIR Gold Nanoparticles.

The fungal culture

The fungal culture yeast and mold cells isolated from the sample. Pure culture is streaked on SDA plate for storing and pure culture is inoculated in SDA broth for antifungal activity test by using MIC agar diffusion method.

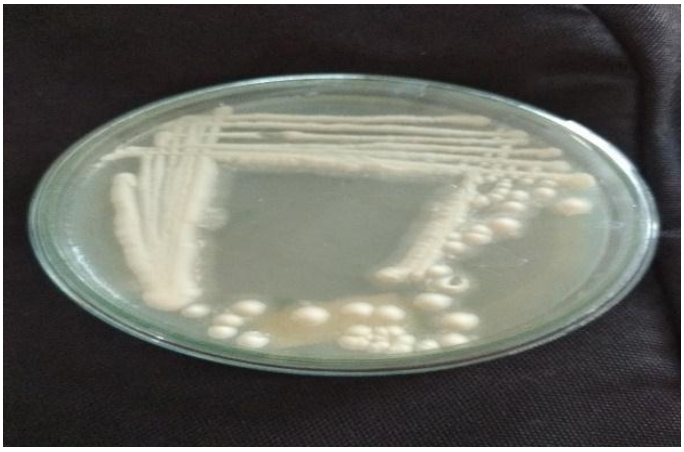


Fig: 5. Candida in SDA plate

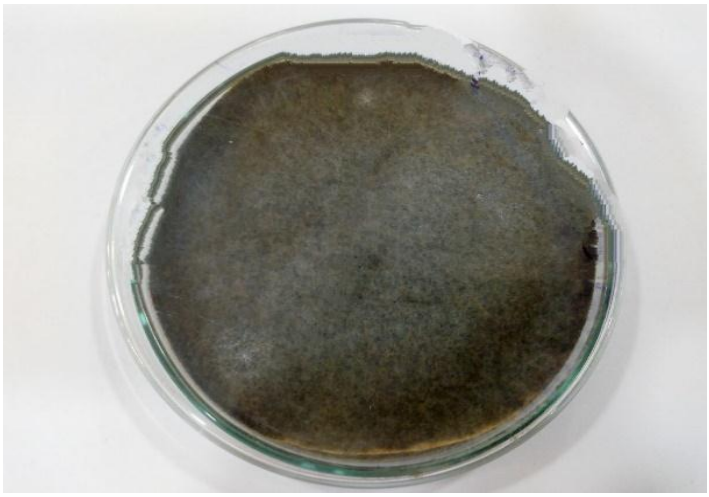


Fig:6 Aspergillus in SDA.

Zone formation

The yeast and mold cells are swab on muller hinton agar. The synthesised gold nanoparticle centrifuged and diluted to added different volume in well. Incubated in room temperature 24-46hrs after measure zone in plate. Candida gives result in 200µl and best in250µl but it resident in 100µl and 150µl. Aspergillus gives result in 100µl and best in 150µl but resist in 200µl and 250µl as shown in table (1).

Table 1: result of zone measure.

S.no	*Organism*	*100µl*	*150µl*	*200µl*	*250µl*
1	*Aspergillus*	8mm	3mm	No zone formed	No zone formed
2	*Candida*	No zone formed	No zone formed	5mm	10mm

Fig 7: Aspergillus and *Candida zone formation.*

CONCLUSION

The *candida* infection is very risky and candle carefully.it affect mostly nosocomial infection. Many antibiotics are available for *Candida* infection but it results become resistant.so, we use honey mediated gold nanoparticle are non-harmful to health. By using this nanoparticle control the growth of fungal Culture. MIC invitro method gives the result to kill fungi and future use to design the drug. Finally use to treat cure the *Candida* and *Aspergillus* infection in humans.

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