

**ANTIMICROBIAL ACTIVITIES OF CRUDE SOLVENT EXTRACT
AND SYNTHESIZED SILVER NANOPARTICLES OF
OPUNTIA FICUS-INDICA L. CLADODES**

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Abstract

Opuntia ficus-indica cladodes was examined for their antimicrobial activities by using crude extract and synthesized silver nanoparticles against human pathogenic microbial strains, namely *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Proteus mirabilis* and *Candida albicans*. All extracts demonstrated moderate activity against the tested microbes in a range between 0.53 and 2.33 cm. The maximum inhibition activity was found to be against *K. pneumoniae* while the lowest against *S. aureus*. Synthesized silver nanoparticles (Ag-NPs) showed inhibition activities between 1.82 and 2.03 cm. The maximum activity was recorded against *K. oxytoca*, while the lowest activities against *P. mirabilis*. In context of antimicrobial activity, there was no significant difference either solvent crude extract or synthesized silver nanoparticles against tested microbes. Therefore, crude extracts of *O. ficus-indica* cladodes or Ag-NPs could be used as an alternative natural drug.

Introduction

Nowadays the multidrug-resistant bacteria becoming a global risk to public health whereas many workers in recent years have focused on how to develop new antimicrobial agents from natural sources for combating such microbial strains (Martin *et al.* 2015). Finding cheaper, having a little side effect than the synthetic chemical alternative agents from plant sources is also other main goal for the researchers (Abouhosseini *et al.* 2012, Dastagir *et al.* 2012). *Opuntia* sp. had been extensively studied for their antiviral, antitumor, cytotoxic, antimicrobial, and for their pesticidal activities (Galati *et al.* 2007, El-Feghali *et al.* 2018). Chemical analysis showed that the cladodes of *O. ficus-indica* consist of polyphenols and vitamins that make it medically significant at the level of antioxidant, antimicrobial, and hypoglycemic and those in turn found to be effective against inflammation, heart problems, vascular disorders, and cancer (Trombetta *et al.* 2006). Previous study showed that the solvents extracted from *O. ficus-indica* cladodes and flowers had high-to-moderate antimicrobial activities against some bacterial and fungal pathogenic species (Ennouri *et al.* 2014, El-Feghali *et al.* 2018). *O. ficus-indica* was recorded in Central America (Mexico) and later, the species was introduced to many areas such as savanna regions inhabiting South Africa, Asia, and Australia. In other parts, it was introduced as an ornamental plant, for camel feeding, and for its delicious fruits as a food for humans. In alternative medicine, the fruits and stems of *O. ficus-indica* provide sources to treat digestive problems such as diabetes and burns (Lee *et al.* 2003).

Opuntia spp. extracts have complex compounds and pure secondary metabolites (Prabhu and Poulouse 2012), hence this property should be exploited to reduce metal ions such as gold and silver into nanoparticles, an important phenomenon termed green biosynthesis. Applications of

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nanoparticles have been succeeded in the medical field study as to aid the diagnosis of cancer as well as developing alternative antimicrobial drugs instead of industrial antibiotics as the resistant microbial strains being identified (Ahmed *et al.* 2016).

The plant distributed widely on Saudi Arabia especially in abandoned area and around the cultivated regions. Details about antimicrobial activities of the cladodes of *O. ficus-indica* invasive plant had been reported very little. Therefore, this attempt aimed at evaluating the antimicrobial activities of *O. ficus-indica* cladodes from either their solvent crude extracts or Ag-NPs extracts could be first report in the pharmaceutical area.

Materials and Methods

Opuntia ficus-indica L. fresh samples were collected in October 2017 from Raydah Reserve Mountains, Kingdom of Saudi Arabia. Some plant parts were kept in plastic bags and preserved in the Refrigerator at 4°C for further use. Three hundred grams of *O. ficus-indica* fresh cladodes were dried for three months under shade in 23°C and then ground to a fine powder by using a mortar and pestle for dry extraction. Fifty grams were crushed directly using an electric grinder to a fine solution for fresh extractions, then filtrated through using Whatman filter paper No. 1..

Five grams of powder from each plant material and mucilage solution were weighed in a test tube. Fifteen ml of the solvent including petroleum ether, 1-butanol, methanol, ethanol, and chloroform were added to each sample. All samples were placed in a rotary shaker at 150 rpm at 37°C for 48 hrs to allow complete extraction of chemical compounds. All solvents in each tube were evaporated using an oven at 59°C for one week. Weighted dry extracts were dissolved in 15 ml of dimethyl sulfoxide (DMSO) and 5 ml for fresh samples and incubated in a rotary shaker at 150 rpm at 25°C to ensure complete dissolving of crude plant extracts (Salvat *et al.* 2004).

The test microbial strains were obtained from the Microbiology Department, Faculty of Medicine, King Khalid University, Kingdom of Saudi Arabia. The strains included one Gram-positive (*S. aureus*), three Gram-negative (*P. mirabilis*, *K. oxytoca* and *K. pneumoniae*) bacteria, and one pathogenic fungus (*C. albicans*). All pathogenic strains were first sub-cultured in nutrient broth (NB) and incubated aerobically at 27°C for 24 hrs for all bacterial isolates and for 48 hrs for *C. albicans*.

Fifty various extracts in triplicate were tested for their antimicrobial activities against *S. aureus*, *P. mirabilis*, *K. oxytoca*, *K. pneumoniae* and *C. albicans*. Twenty ml from a previously made sterilized nutrient agar (NA) medium was transferred into sterile Petri plates and left for one hour at room temperature to allow for solidification. A total of three wells were made in each nutrient agar plate using a sterile cork-borer (6 mm in diameter). Each microbial strain of 0.1 ml was spread over the solid agar using a sterilized loop. One hundred µl of the prepared plant extract was transferred into the wells using a sterilized pipette. The plates were then incubated at 37°C for 24 hrs. The diameters of the inhibition zone in centimeters were measured using a transparent ruler. Each experiment was repeated three times and the mean values were calculated (Moustafa *et al.* 2013).

Five grams of the cladodes from *O. ficus-indica* was transferred into 50 ml of deionized water (DIW). All samples were placed into a rotary shaker at 150 rpm at 25°C for three days to allow complete extraction. Extracts were filtered using Whatman filter paper No. 1 in a conical flask. The conical flask containing 25 ml of 0.167 mM AgNO₃ was heated at 70°C and the plant extracts were added drop by drop until the colloidal solutions turned yellow for the first ten minutes and turned dark brown after one hour and then kept at 4°C for further use. The absorbances of colloidal solutions were measured with a spectrometer (Beckman - Model No. DU-50, Fullerton, CA, USA). One ml of the suspension was collected to evaluate the completion of Ag⁺ in aqueous

solution, then diluted with an appropriate amount of deionized water and subsequently observed in the UV-visible spectra, between wavelengths of 400 and 700 nm. Images of Ag-NPs were obtained using a scanning electron microscope (SEM; Hitachi S4800) equipped with an energy-dispersive X-ray spectroscope.

Antimicrobial activities of solutions containing nanoparticles synthesized from cladode part of *O. ficus-indica* were performed against various microbial strains. Twenty ml of the sterilized nutrient agar (NA) medium was transferred into sterile Petri plates and five wells were made. 0.1ml inocula were spread over the solid agar and then 100 μ l of Ag-NPs was filled in each well. Each extract was repeated three times and mean values were recorded. DMSO was used as a negative control while cefoxitin (30 μ g) dissolved in 15 ml DMSO was used as positive control. The generated data were analyzed using Graph Pad Prism 6 Demo (version 7.04).

Results and Discussion

This study achieved the synthesis of silver (Ag) nanoparticles from silver nitrate using the cladode extract powder of *Opuntia ficus-indica*. Addition of *O. ficus-indica* cladode extract to 0.167 mM AgNO₃ aqueous solution led to the appearance of a dark brown color in the solution after approximately 1 hr of reaction, indicating the formation and stabilization of silver nanoparticles (Fig. 1). The silver nanoparticles appeared to be a reddish-brown color in water. This color may be due to excitation of surface plasmon vibrations in the silver nanoparticles, as mentioned in other study (Song and Kim 2009). The UV-vis spectra showed the maximum absorbance in range 420-470nm (Fig. 2). Moreover, a SEM/EDX analysis confirmed the elements present in the solution at the rate of Ag (74.33%), C (8.09%) and Cl (11.95%) (Fig. 3). In addition, *O. ficus-indica* cladode extract consists of many phytochemicals that play an important role in reducing ions to form nanoparticle sizes. The carboxylic group significantly affected the surface of the nanoparticles, and controlled the stabilization process through electrostatic forces (Prabhu and Poulouse 2012). The antimicrobial activity of extracts of *O. ficus-indica* cladode was tested against four pathogenic bacteria, and one pathogenic fungus. The study revealed variations in antimicrobial activities by applying various solvent plants extracted (dry and fresh extracts (Figs 4 and 5). DMSO, which was used as a negative control, demonstrated no inhibiting effects. The positive controls showed inhibition diameters ranging from 2.00 ± 0.02 - 2.5 ± 60.04 cm (Cefoxitin dissolved in DMSO). The findings of this study showed that solvent extracts of fresh cladode of *O. ficus-indica* including those from 1-butanol, methanol, and chloroform had significant antimicrobial properties (Fig. 5). On the other hand, the maximum inhibition effect of dry cladode of *O. ficus-indica* extracts were from ethanol, petroleum ether, chloroform, and 1-butanol extracts (Fig. 4). It is clear that the effectiveness of plant extract is widely affected by the type of solvent. In this study, both organic solvents (polar and non-polar) stimulate precipitation of active chemical compounds with varying relative effectiveness. They showed a moderate-to-slight activity against pathogens that varies from one sample of *O. ficus-indica* to another. Related results demonstrate that the organic extract has significantly varying polarity impact on tested microbes (Sen and Batra 2012).

The results of the antibacterial screening illustrated that all solvents of dry cladode extracts have inhibitory effects against *K. pneumoniae* and all solvents of fresh extracts have inhibitory effects against *C. albicans*. Petroleum-ether dry extracts exhibited a higher degree of antimicrobial activity as compared to other solvent extracts (2.33 ± 005 cm) followed by the methanol dry plant extract (1.76 ± 0.11 cm). In contrast, the 1-butanol dry extract activity recorded the lowest degree of such activity (1.10 ± 0.1 cm) (Fig. 4). Additionally, chloroform and methanol fresh extracts of *O. ficus-indica* cladode displayed promising activity against *K. pneumoniae* (Fig. 5). The extracts of

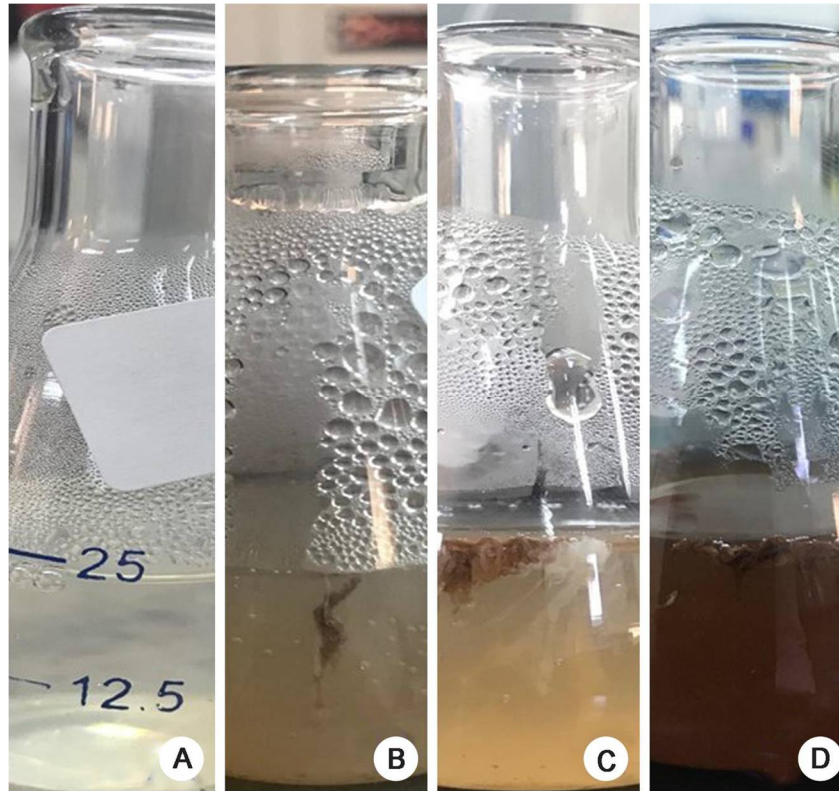


Fig. 1. Synthesis of silver nanoparticles (A) AgNO₃ solution, (B) after 10 min synthesized Ag-NPs in yellow-color solution, (C) after 30 min turned to brown color solution and (D) after 1 hr formation and stabilization of Ag-NPs in brownish red solution.

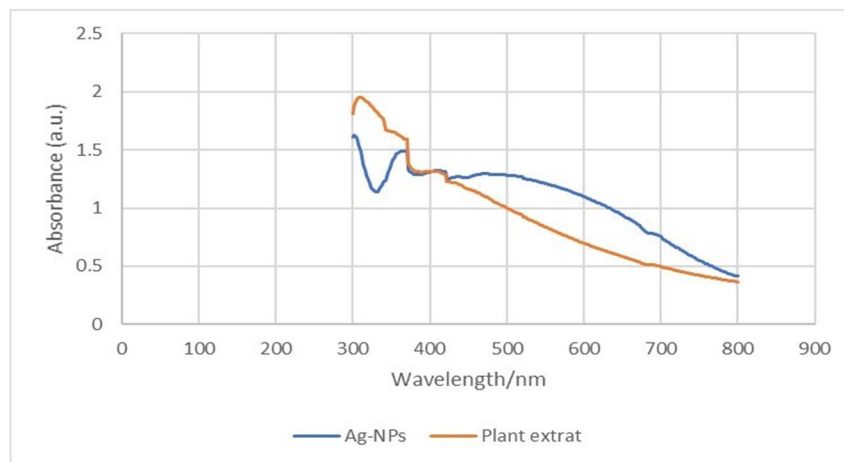


Fig. 2. UV-visible spectra of Ag-NPs solutions synthesized from *O. ficus-indica* cladode extract.

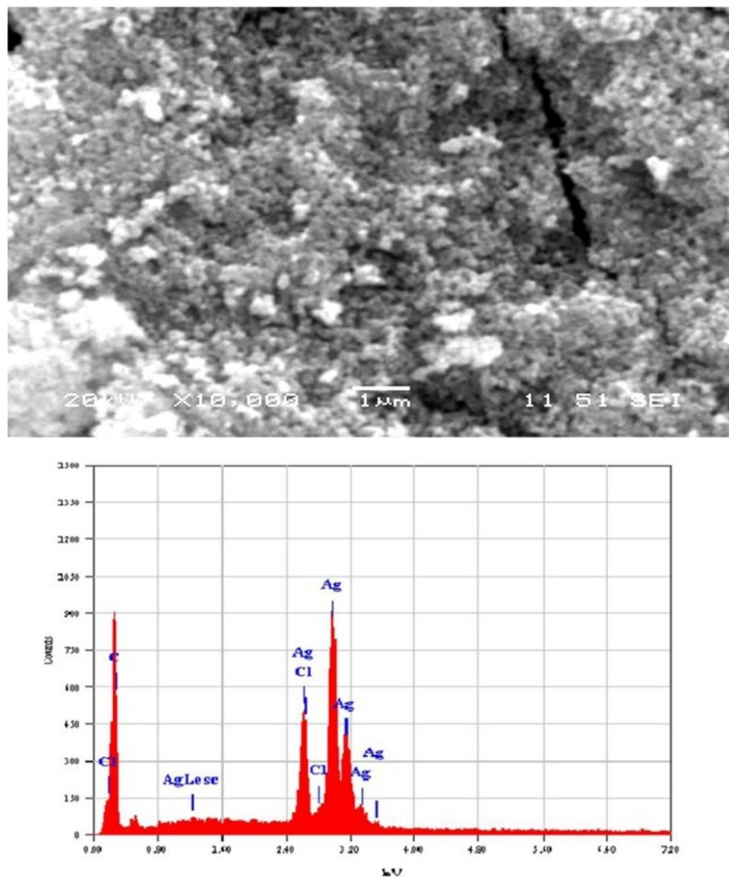


Fig. 3. SEM images of Ag-NP solution synthesized from *O. ficus-indica* cladode and corresponding EDX.

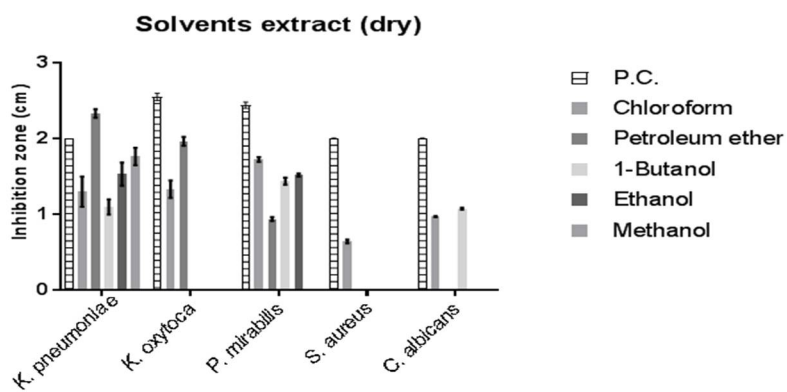


Fig. 4. Antimicrobial activity of *O. ficus-indica* dry cladode extracts against pathogenic strains.

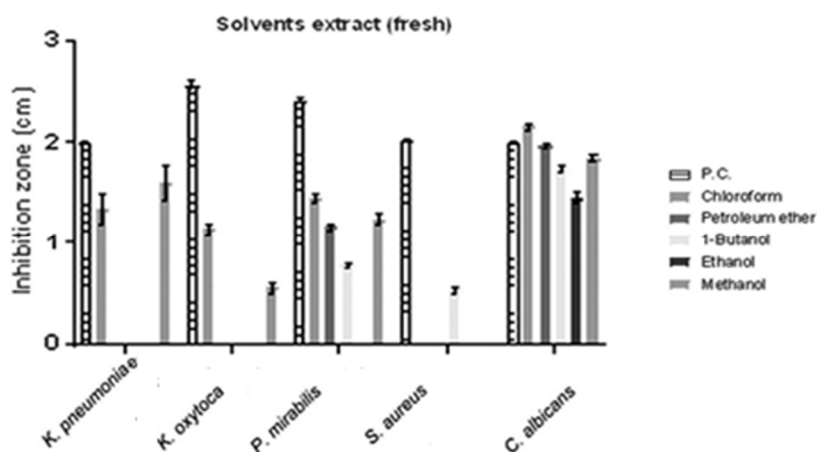


Fig. 5. Antimicrobial activity of fresh cladode extracts of *O. ficus-indica* against pathogenic strains.

the dry and fresh plants tested were found to be active against *K. oxytoca*. Fig. 4 clearly illustrated that the solvent dry extracts from cladode of *O. ficus-indica* had a narrow antibacterial activity. The highest inhibitory activity was recorded from the petroleum ether and chloroform dry extracts (1.96 ± 0.05 and 1.33 ± 0.11 cm). Fig. 5 illustrates the promising inhibition activity against *K. oxytoca*, which was gained only from chloroform, and methanol fresh extracts of *O. ficus-indica* cladode (1.13 ± 0.05 and 0.55 ± 0.04 cm). In case of antibacterial activity against *P. mirabilis*, the highest antibacterial potential observed from plant dry extract are as follows: chloroform dry extract represented the highest activity (1.76 ± 0.03 cm), followed by ethanol and 1-butanol extracted (1.52 ± 0.02 and 1.44 ± 0.04 cm). However, petroleum ether dry extract exhibited less antibacterial activity (0.94 ± 0.02 cm). The results also demonstrated that solvents extracted from the fresh cladode of *O. ficus-indica* had less activity than dry extract and the positive control, which had a narrow spectrum against *P. mirabilis*. Chloroform fresh extract exhibited the maximum activity (1.44 ± 0.04 cm), followed by methanol (1.24 ± 0.04 cm), while 1-butanol extract showed the minimum value of inhibition zone (0.76 ± 0.02 cm). 1-butanol and chloroform extracts from the dry cladode showed potential of anticandidal activity against pathogenic *C. albicans* (1.07 ± 0.01 and 0.97 ± 0.01 cm). On the other hand, all extracts from *O. ficus-indica* fresh cladode have inhibitory effects against the tested *Candida*. The strongest spectrum of activity attained from the chloroform extract (2.15 ± 0.03 cm), which was greater than the positive control (Cefoxitin dissolved in DMSO) and other solvents. However, ethanol extract recorded the minimum activity (1.45 ± 0.04 cm).

This difference in activity between different organic extracts may be due to the variations between extract compounds in the fresh and dry cladode of *O. ficus-indica* extract. This finding is in general agreement with the result of Cowan (1999). Preethi (2010) mentioned that the alcoholic extract had a high degree of antimicrobial activity in different medicinal plants while petroleum ether extract exhibits intermediate and aqueous extract showed a low degree of antimicrobial activity. In addition, no stable chemical compound or volatile oil may be changed through drying process, such as the fresh samples containing the mucilage layer that coating polysaccharides with high molecular weights that are difficult to dissolve in water or may be in other solvents. Furthermore, perhaps the time of collection and storage conditions could have contributed to the

contrasting results (Olafimihan 2004). Existence of polyphenols (limited solubility in water), calcium oxalate, and compounds of flavonoids was found to have antimicrobial activity (El-Feghali *et al.* 2018).

Fig. 6 showed antimicrobial activity with Ag-NP solutions against pathogenic bacteria and pathogenic candida. The antimicrobial activity of Ag-NP solutions against *K. pneumoniae* and *C. albicans* comprised of the widest spectrum of all pathogens (2.03 ± 0.05 cm). Contrarily, *P. mirabilis* was the lowest value (1.82 ± 0.02 cm), compared to the positive control (Cefoxitin dissolved in DMSO). In this work, slight susceptibility differences between pathogenic microorganisms were observed along with a significant inhibitory effect of Ag-NP solutions. The findings of this study are not in agreement with the results obtained by Metwally *et al.* (2018),

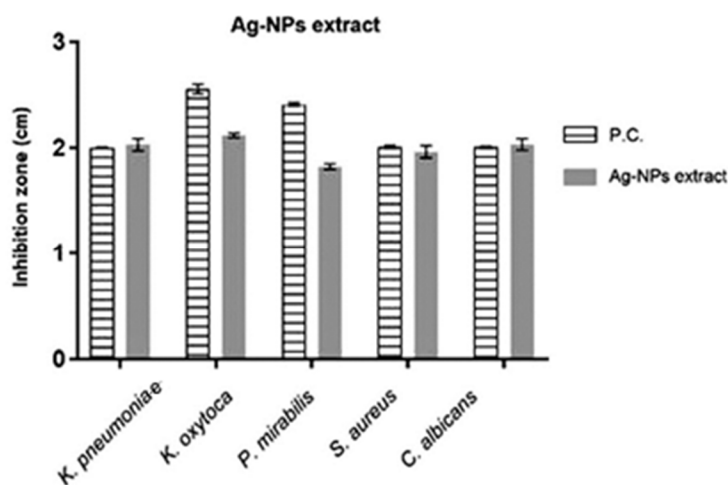


Fig. 6. Antimicrobial activity of silver nanoparticles synthesized from *O. ficus-indica* cladodes extracts against pathogenic microorganisms.

who reported that Gram-negative bacteria was less affected by silver-NP extracts than Gram-positive bacteria due to the positive charge of silver NPs that interact with the Gram-negative lipopolysaccharide. This result again supports that in the extract of *O. ficus-indica* plants, there were specific chemical compounds targeted to the positive bacterial strains. Petica *et al.* (2008) have reported that silver nanoparticles exert the same effect on Gram-positive and Gram-negative bacteria and a fungal mix of *Aspergillus*, *Penicillium* and *Trichoderma* species. This study also reported that Gram-positive bacteria (*S. aureus*) are more resistant to plant extracts than Gram-negative bacteria (*P. mirabilis*, *Klebsiella* spp.), which were inhibited by most *O. ficus-indica* dry and fresh cladode extracts. The Gram-negative hydrophilic surface of the bacterial cell wall is thought to act as a barrier to many unusual substances, including antibiotics (Nikaido 1994). It is speculated that particular chemicals may be working specifically for Gram-positive rather than -negative bacteria.

These results indicated that there is a considerable difference in antimicrobial activity between solvent extracts and Ag-NP solutions. The Ag-NP solution showed that there is hyper-inhibitory activity against tested microbes that differs from extract of cladode for all solvents whereas extracts showed a high degree of specific antimicrobial activity against *C. albicans* and *K. pneumoniae*. The difference is due to the nanoparticles having large surface areas, which allow them to penetrate the pathogenic cell wall more easily by attaching Ag-NPs to its cell membrane,

interact with it, then enter and damage the cell. This mechanism indicates the efficiency of Ag-NPs antimicrobial activity properties as confirmed by other researchers (Morones *et al.* 2005).

These solvent extracts and synthesized AgNPs obtained from *O. ficus-indica* cladodes which proved to be potentially effective against human pathogenic microbes could be used as natural alternative drugs for microbial control than synthetic chemicals.

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