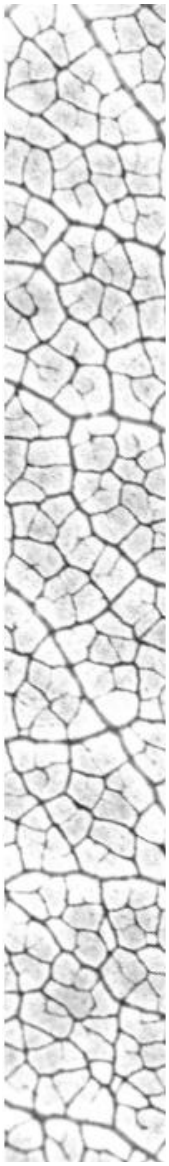

EurAsian Journal of BioSciences
Eurasia J Biosci 14, 4829-4835 (2020)

Phytochemicals screening and antibacterial activity of silver nanoparticles, phenols and alkaloids extracts of conocarpus lancifolius

Rana Hussein Raheema [1*], Roaa M. H. Shoker [2]

[1] Department of Medical Microbiology, Faculty of Medicine, University of Wasit, IRAQ
[2] Department of Pharmacology, Faculty of Medicine, University of Wasit, IRAQ
*Corresponding author: rraheema@uowasit.edu.iq

Abstract
The emergence and spread of microbial drug resistance and lack of developing new antibacterial bacteria are a growing problem in multi-drug - resistant bacteria, Phytochemical compounds and silver Nanoparticles (NPs) synthesis has been of great interest because of their potential biomedical applications. Hence this study was designed to evaluate the effect of Conocarpus lancifolius extracts s to control multidrug-resistant bacteria. Chemical detection of aqueous extracts of Conocarpus lancifolius leaves revealed the presence of tannin, Saponins, Comarins, phenols, alkaloids, flavonoids, glycoside and terpenes compounds. Results showed yield of phenols extracts of C. lancifolius were 46.1% while yield of extracts 22.2% alkaloids. AgNPs was proved by Atomic Force Microscopy. The average diameter of 75.50 nm. HPLC analysis indicated the presence of four phenolic compounds were Rutin, Epigenen and Kamferol and Catechine while contains two alkaloids Scopolamine and Hyoscine. Results showed that Conocarpus lancifolius extracts and AgNPs, possess higher antibacterial activity against both gram-positive and gram-negative pathogenic bacteria

Keywords: *Conocarpus lancifolius*, phytochemical Screening, AgNPs, AFM, HPLC, antibacterial activity

Raheema RH, Shoker RMH (2020) Phytochemicals screening and antibacterial activity of silver nanoparticles, phenols and alkaloids extracts of conocarpus lancifolius. Eurasia J Biosci 14: 4829-4835.

INTRODUCTION

Nearly 50% of the worldwide hospital-acquired infections are caused by multidrug-resistant (MDR) microorganisms. In addition to vancomycin-resistant Enterococci (VRE) and Mycobacterium tuberculosis, penicillin-resistant Streptococcus pneumoniae, Shigellas and Salmon, a few strains of particular concern include bacteria developing extended-spectrum beta-lacktamases (ESBL) including Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae or Acinetobacter baumanii and Helicobacter pylori (Sibanda and Okoh, 2007; Abreu et al., 2012). Scientists and clinicians around the world are interested in discovering new bioactive components in plants. Plants are the principal sources of various biomedical applications of different forms of plant chemicals. In most advanced sciences, medicine and technologies, nanotechnology is an increasingly rapid developing field and has a significant role in that area (Kumar etal.,2015 and Rajeshkumar,2018). Continuing to use traditional medicine is not only because of culture and poverty but also because many modern drugs are ineffective (Tenover, 2006). The inefficiencies of successful remedies and the resistance to new herbal therapeutic agents produced by the present antibiotic pathogens (Percival, 1997 & Berrino et al., 2009). In herbal medicine, C. Lancifolius has many applications, including anaemia, catarrh, conjunctivitis, diabetes, diarrhoea, vomiting, haemorrhage, orchitis, skin ulcers, and syphilis (Liogier, 1990 and Morton, 1981; Asfaw and Fentahun, 2020). C. lancifolius is a fast-growing and dry tolerant tree species with potential for phytoremediation in the arid climate (Rasheed etal.,2019). Conocarpus lancifolius is a Somali-born common riverine tree. Initially restricted to a small area along the outside Red Sea coast, it spread over the past decade to the south and middle of Iraq because of its ability to develop in extreme conditions (daily temperature more than 40 C, water inadequacy and salinity). Conocarpus has now become Iraq's largest perennial decorative tree.

Received: July 2019
Accepted: March 2020
Printed: October 2020

The evergreen tree quickly spreads and can generate a significant amount of biomass with gout irrigation (Suleman et al., 2013) and (Ali, 2014). The present work aims to study the antibacterial activity of aqueous, crude phenols, crude alkaloids and silver nanoparticles extracts of Conocarpus lancifolius against gram-positive and negative bacteria from different clinical diseases.

MATERIALS AND METHODES

Collection and extraction of plant material

Fresh leaves of C. lancifolius were collected from the gardens of Al-kut city and then washed with tap water, and they were shad-dried and then they were grinded. The plant was kindly identified and authenticated by Dr.Sukeyna Abaas Aliwy at the Department of biology / college of science/university of Baghdad. Preparation of different extracts of Conocarpus lancifolius such as prepare an aqueous extract by (Zheng Mu et al., 1990), Crude Phenols extract were extracted according to Ribereau-Gayon (1972) and crude alkaloids were extracted according to Harborne (1984). Different concentrations were prepared 100, 150, 200 mg /ml of aqueous extract and 5,10, 20 µg / ml of AgNPs, alkaloids, phenolic extracts. Chemical detection of aqueous extract of C. lancifolius were tested for the presence of several phytochemicals such as tannins (Shihata, 1951), saponins (Shihata, 1951),coumarins (Geisman, 1962), phenols (Harbone, 1973), alkaloids (Harbone, 1973), flavonoids (Jaffer et al., 1983),glycoside (Evans, 1999) and terpenes and steroid (Al-Abid, 1985).

Green Synthesis of silver nanoparticles

For AgNPs synthesis aqueous solution (1 mM) of silver nitrate ($AgNO_3$) was prepared and used. 5 ml plant extract applied to 1 mM silver nitrate solution for the reduction of Ag^+ ions in 95 ml of solution. The colour shift from colourless to brown has confirmed the removal of silver nitrate to silver ions (Veerasamy et al., 2010). Concerning the preparation of AgNps, 10 mL aqueous extracts were dissolved in 500 mL double-distilled water and boiled for 7–8 min before decantation. A volume of 10 ml of the resultant supernatant was mixed with 50 mL of 1 mM $AgNO_3$. This reaction mixture was incubated until the colour changed to dark brown at room temperature (Veerasamy et al., 2011).

Measurement devises AgNps

Atomic Force Microscopy

Atomic Force Microscopy (AFM) research is a very high-resolution method of scanning probe microscopy, with a validated resolution of fractions of a nanometer. Samples were imaged using the AFM communication mode by Angstrom Advanced Inc. USA, 2008. The surface morphology of nanoparticles was visualized by the Atomic Force Microscope (Veeco) under standard atmospheric conditions. The samples analyzed were scattered on a small slide and explored the communication mode of the instrument (Oliveira et al., 2005). Prepare three concentrations of silver nanoparticles 5, 10, 20 µg / ml.

Fig. 1. Nanoparticle samples. Brown:C. lancifolius aqueous extract. Black: C. lancifolius NPs aqueous extract

Analysis of the C. lancifolius extracts by HPLC

The phenolic compound analysis was performed by injection of 100 µl of an extract of each sample into the High-Performance Liquid Chromatogram (HPLC model SYKAM Germany) for identification. The protocol outlined by Mradu et al., 2012. The analysis was performed in the laboratories of the Ministry of Science and Technology.

Bacterial isolates

ten isolates of gram-positive and negative bacteria were subjected to Conocarpus lancifolius extract, the isolates have been obtained from different clinical diseases in Al-Karama Educational Hospital, Al-Kut city, Wasit province, Iraq and all samples were identified by routine, conventional methods in the microbiology laboratory of medicine college, Wasit university

In-vitro Antibacterial Activity

In-vitro Antibacterial Activity of alkaloids, AgNPs, Phenols and aqueous extracts of C. lancifolius.Agar well diffusion method was used to screen the activity of plant extracts in vitro and different concentration alkaloids, AgNPs, Phenols and aqueous extracts were poured into the wells with DMSO as a control.

STATISTICAL ANALYSIS

Data analysis was performed using SAS (Statistical Analysis System - version 9.1). The association were examined using Chi-square test. Level of significant as $P < 0.05$.

RESULTS AND DISCUSSION

Results of chemical detection of aqueous extracts of C. lancifolius leaves revealed the presence of tannin, Saponins, Comarins, phenols, alkaloids, flavonoids, glycoside and terpenes compounds as a major constituents and absence of steroid as shown in **Table 1**. The results of the present study agree with other research conducted by (Saadullah et al., 2014) who

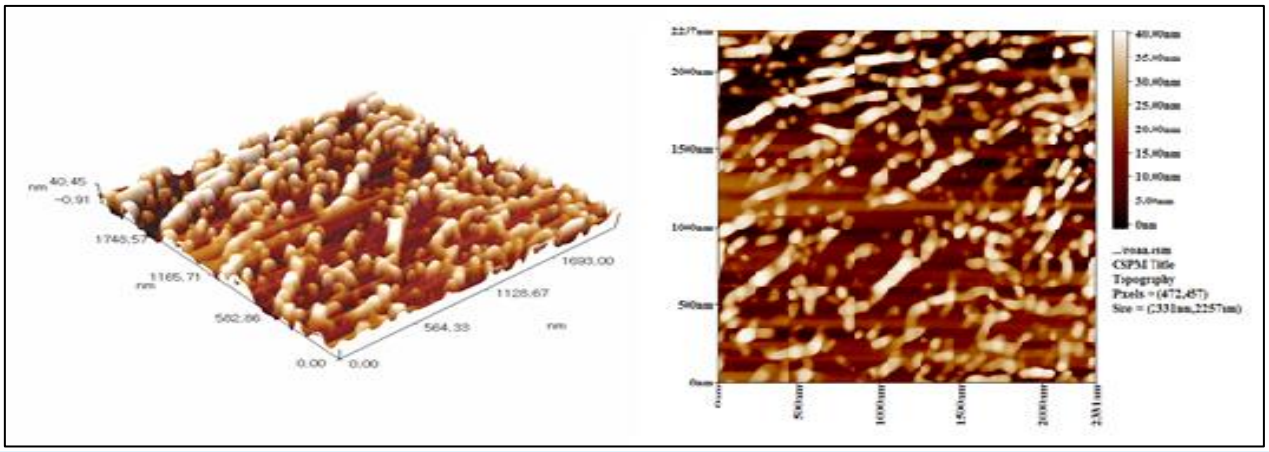

Fig. 2. Topography and three-dimensional images showing the globally spherical shape of the prepared particles of *Conocarpus lancifolius*

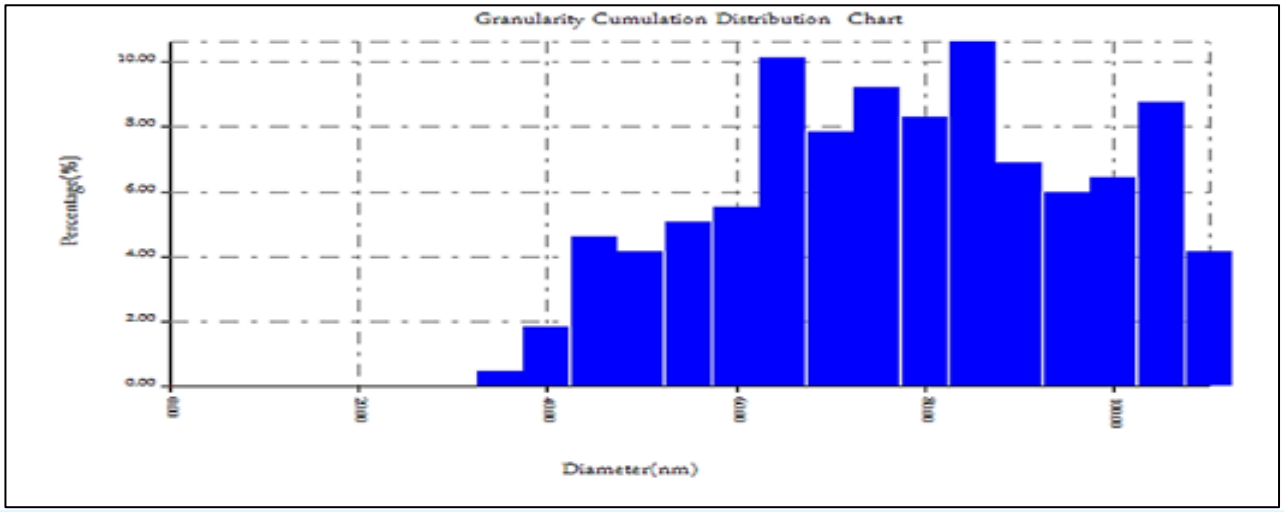

Fig. 3. Granularity distribution chart obtained from AFM software of *C. lancifolius*

Table 1. Single Chemical test of some active compounds in *C. lancifolius* aqueous extracts

Active Secondary compounds	Test name	C. lancifolius Aqueous extracts
Tannins	Detection of Tannins	+
Saponins	Detection of Saponins	+
Comarins	Detection of Comarins	+
Phenols	Detection of Phenols	+
Alkaloids	Detection of Alkaloids	+
Flavonoids	Detection of Flavonoids	+
Glycoside	Detection of Glycoside	+
Terpenes	Detection of Terpenes	+
Steroid	Detection of Steroid	-

Table 2. Yield of *C. lancifolius* extracts expressed as %

Plant Name	extract Type	Yield (%)
C. lancifolius	Phenols	46.1 %
	Alkaloids	22.2 %

Table 3. Ag NP diameters synthesized of *C. aqueous* extracts

No	Nanoparticle solutions	Nanoparticle diameters (nm)		Average
		50%	90%	
1	Ag NPs with C. lancifolius aqueous extracts	75.00	100.00	75.50

mentioned that C. lancifolius contained glycosides, tannins, saponins and terpenoids.

Results showed a yield of phenols extract of Conocarpus lancifolius were 46.1%, while yield of alkaloids extract 22.2%, **Table 2**. Result of silver nanoparticles revealed the colour change was due to the surface resonance phenomenon, the growth of solution colour during synthesis and the formation of the yellowish-white precipitate suggested a reduction in zinc nitrate. Atomic force microscopy was one of the primary tools for measuring, imaging and manipulating matter at the nanoscale. It was employed to characterize the size and morphology of AgNPs. **Fig. 2** showed AFM images and corresponding size distribution of prepared AgNPs, and it was found that the average diameter of about 75.50 nm. Nanoparticles are particulates of less than 100 nm at least one scale (Vidya et al., 2013).

Granularity distribution chart was obtained from AFM software for biological methods which gives the particle size distribution of Ag NP, **Fig. 3**, **Table 3**. Many plant parts and extracts showed the ability to synthesize

Table 4. Types and concentration of phenols extracts in plant

Phenolic compounds	Concentration (µg/ml)
Rutin	624.28
Epigenen	348.81
Kamferol	48.44
Catechine	96.41
Total concentration (µg/ml)	1117.94

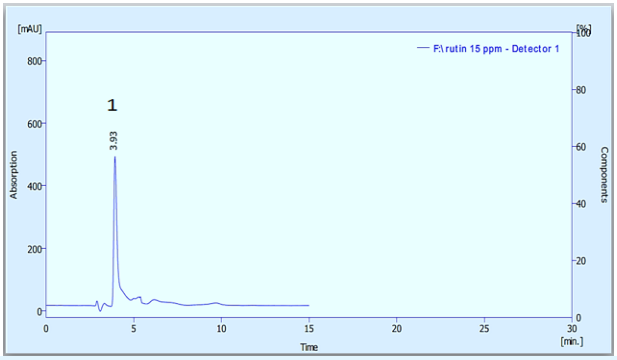

Fig. 4. HPLC profile of phenolic stander (1-Rutin)

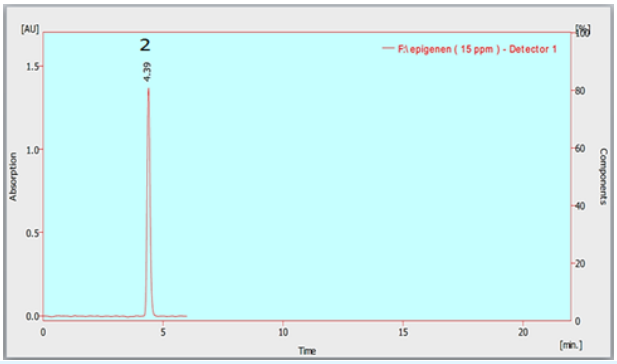

Fig. 5. HPLC profile of phenolic stander (2-Epigenen)

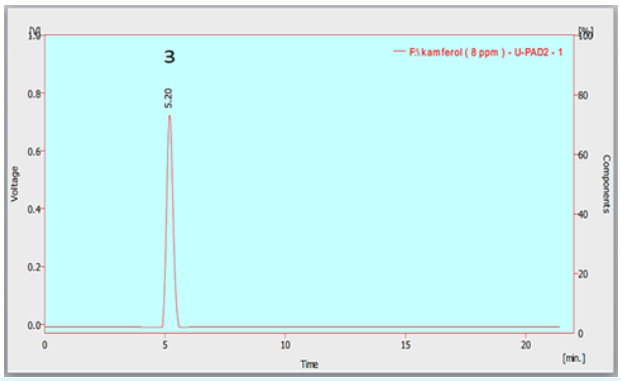

Fig. 6. HPLC profile of phenolic stander (3- Kamferol)

nanoparticles compared to the other study for plant latex extracts by (Saleh and Najim.,2020) revealed the production of AgNPs in different sizes of 103 and 82 nm using the Moraceae family and 77 and 74nm using the Euphorbiaceae family.

The findings of the analysis are in accordance with Klaus et al. (1999), who noted the use of AgNPs as a reducer agent as an effective early agent technique. Ag NPs are widely used in soap, detergents, detergents,

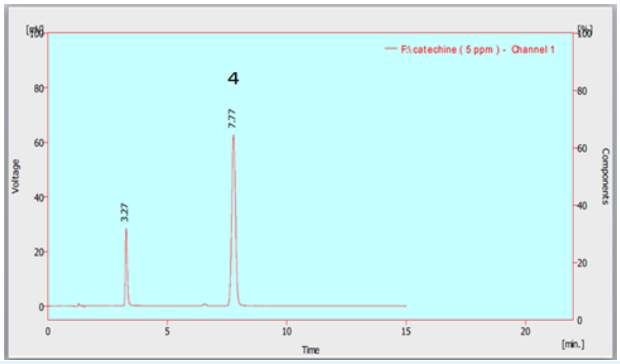

Fig. 7. HPLC profile of phenolic stander (4-Catechine)

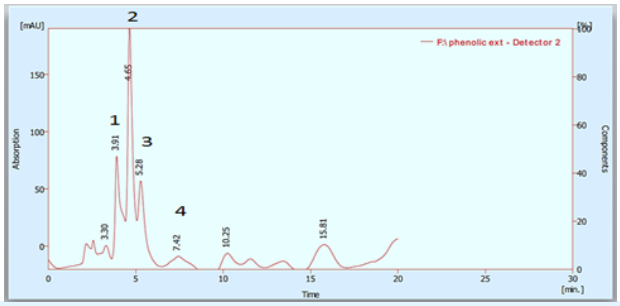

Fig. 8. HPLC profile of Phenolic plant extract (1-Rutin, 2-Epigenen, 3-Kamferol and 4-Catechine)

dentures, cosmetics, medications, vitamins and devices and medicinal products, and are also directly related to human life (Pileni, 2000). Ag NPs are also commonly used in soap products. Those findings were in accordance with Nair and Pradeep, 2002; Willner et al. (2006), who found that unsustainable demand and existing market demand have been overcome and the labour force or production of dangerous materials has fallen for human life and the environment concerned.

The essential phenol constituent was Rutin (624,28 µg / ml), Epigenen (348,81 µg / ml) and Kamferol (84,44 µg / ml), while Catechin (96,48 µg / ml) was minor in HPLC analyzed findings suggesting the existence of 4 phenolic compounds in C. lancifolius. **Table 4** and **Figs. 4-7** indicate the compound concentration. The redox processes are known to be phenolic compounds like tannins, flavonoids, and phenolic acids. Hence they play a crucial role in decreasing or decreasing the activity of drug plants, fruits or vegetables. The phenolic compounds are hydrogen donors, reducers, single oxygen sprinklers, and even metal chelating agents because of their redox activity (Rice-Evans, 2004)..There are several kinds of phenolic compounds in plant extracts. These compounds are highly reactive and participate in redox reactions. For reduction of the metal ions and formation of nanoparticles of the respective metals, the presence of complete phenolic materials in the plant extract may be liable (Nasrolllahzadeh and Sajadi 2015). The operation of phenolic antioxidants is useful as a part of anti-ageing and cosmetic products (Boudet, 2007) for therapy and

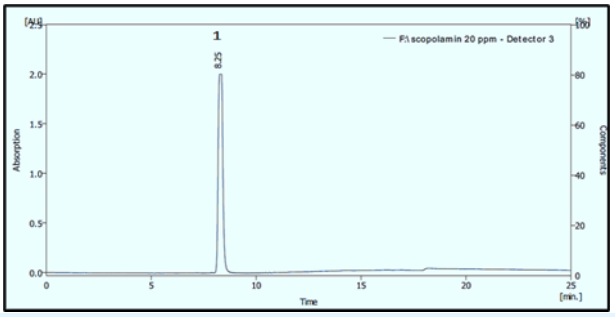

Fig. 9. HPLC profile of alkaloids stander (1-Scopolamin)

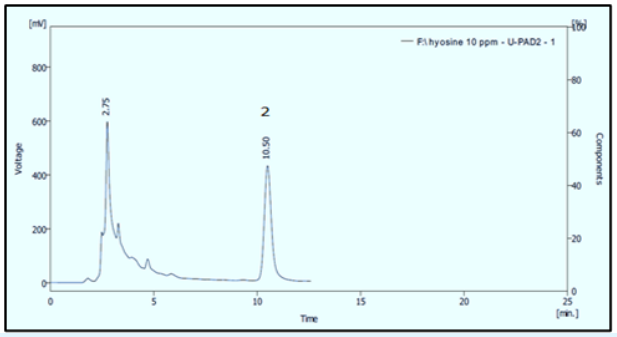

Fig. 10. HPLC profile of alkaloids stander (2-Hyoscine)

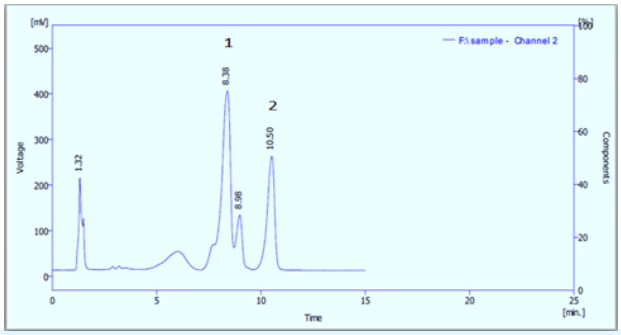

Fig. 11. HPLC profile of plant alkaloids Extract (1-Scopolamin, 2- Hyoscine)

the prevention of cardiovascular, cancer and neurodegenerative illnesses.

Results showed that Conocarpus lancifolius contained 2 alkaloids, while Scopolamin (1218.678µg / ml) was the main alkaloid compounds, while Hyoscine (699.242µg / ml) was the minor. **Fig. 6** and **9**, **10** and **11**. Antibacterial activities of alkaloid, silver nanoparticles, phenol and aqueous extracts of Conocarpus lancifolius at different concentration (5, 10, 20 µg/ml) for alkaloid, silver nanoparticles, phenol, respectively and (100, 200, 300 mg/ml) for aqueous extract against both bacteria and fungi were studied. **Fig. 12**.

Results revealed that the differences among the zone inhibition for the different types of bacteria were significant ($P<0.05$) the highest zone inhibition for the alkaloid extract was shown in the Proteus mirabilis for all concentrations and the lowest for Bacillus cereus and Staphylococcus capitis. Also, results showed that the differences in the zone of inhibition of Bacillus cereus for different concentrations of all groups were significant ($P<0.05$), the highest mean was detected in phenol extract (20 mg/ml) while the lowest was shown in alkaloid group (5 mg/ml). For silver nanoparticles extract, the highest mean was detected in (20 mg/ml) for MRSA and staphylococcus epidermedius while the lowest as shown in (5 mg/ml) for staphylococcus cohnii. The highest zone inhibition for the phenol extract was demonstrated in the staph aureus for all concentrations, and the lowest for pseudomonas aeruginosa. For aqueous extract, the highest zone inhibition was demonstrated in the MRSA and Proteus mirabilis for all concentrations and the lowest for pseudomonas aeruginosa. The results in this study were in agreement with Ali et al. (2013), who mentioned the current work has led to the alkaloids extract of C. lancifolius leaves

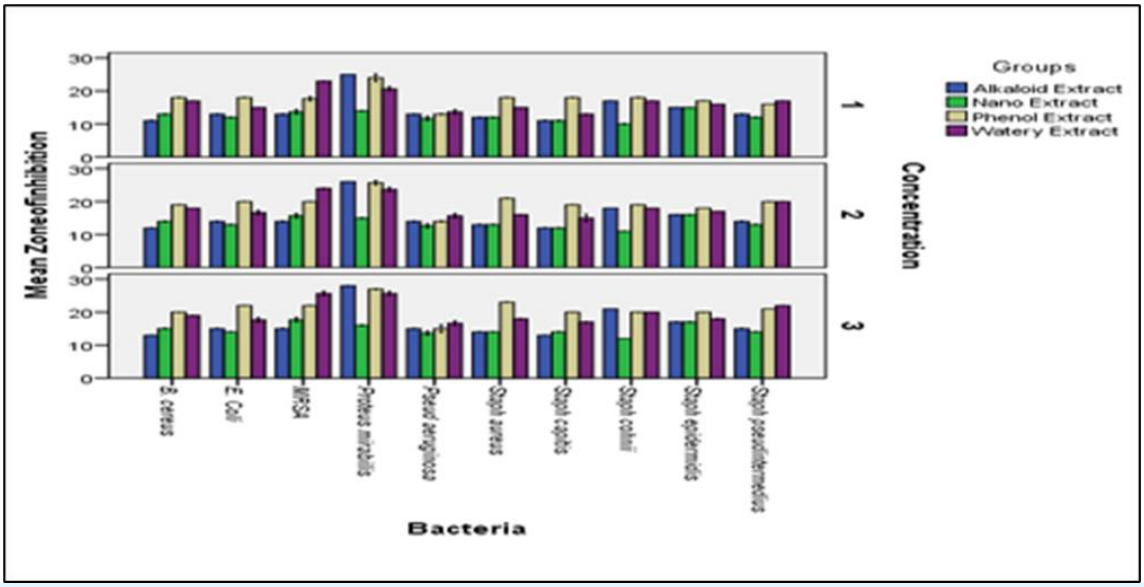

Fig. 12. Effect of alkaloids, AgNPs, phenolic and aqueous extracts of C.lancifolius on the bacteria isolates at different concentrations

exhibiting relevant antibacterial activity. The medicinal plants such as C. lancifolius can be promising sources of potential antibacterial. Also similar with a study by Hussein, 2016 who confirmed that Conocarpus leaves extract could be utilized to preclude damage produced via free radicals and infections produced via pathogenic bacteria, this extract had the highest antioxidant capacity and antibacterial activity growth of some bacteria such as Staph. aureus, Straptococcus, E.coli, Enterobacter. Raheema, 2016 revealed the extract of the plant have antibacterial and protective roles against pathogenic bacteria. The outcome is not in accordance with this analysis of Saad et al., 2014, who found that the plant possesses moderate antibacterial activity and low antifungal activity.

CONCLUSION

In conclusion, the findings indicate that Conocarpus lancifolius antibacterial activity in various pathogenic strains is antibacterial, silver nanoparticles, phenol and aqueous extract. Additional studies on the in vivo antibacterial function need to be performed in the future.

ACKNOWLEDGEMENTS

We would like to express our heartfelt thanks to department of Medical Microbiology, Faculty of Medicine and department of Pharmacology, Faculty of Medicine, University of Wasit for their assistant and effort during study.

REFERENCES

Abreu, A. C., McBain, A. J., and Simoes, M. (2012). Plants as sources of new antimicrobials and resistance-modifying agents. Nat. Prod. Rep. 29, 1007–1021. doi: 10.1039/c2np20035j

Al-Abid, M. R. 1985. Zurr Zusamme mesturungder absuchla B membrane in Phoenix datylifera. Wurzburg University.Wurzburg F.R. of Germany. (Cited by Al-Zorri S. (2009).

Ali H. M., Salem M. Z., Abdel-Megeed A. (2013). In-vitro antibacterial activities of alkaloids extract from leaves of Conocarpus lancifolius Engl. J. Pure Appl. Microbiol 7, 1903–1907.

Ali, B.A.A., Resistance of Conocarpus Lancifolius Engl. to Different Levels of Salinity and Water Supply, ARPN Journal of Agricultural and Biological Science, 9(6), pp. 211-215, 2014.

Asfaw M and Fentahun T (2020). Treatment trials of epizootic lymphangitis with local medicinal plants: a review. Online Journal of Animal and Feed Research, 10(4): 158-161.

Boudet A.M (2007).Evolution and current status of research in phenolic compoundsPhytochem6822–24200727222735

Evans, W.C. 1999. Trease and Evans Pharmacognosy. 14th ed. W.B. Saunders company Ltd. UK.

F. Berrino, A. Verdecchia, J. M. Lutz, C. Lombardo, A. Micheli, and R. Capocaccia, “Comparative cancer survival information in Europe,” European Journal of Cancer, vol. 45, no. 6, pp. 901–908, 2009.

Giesman, T.1962.Chemistry of Flavonoids Compounds.Macmillan Co. New York.

Harborne, J. B. 1973. Phytochemical methods.2nd ed. Champan & Hall, London. Pp: 159-165.

Harborne,J.B.1984.Phytochemical methods. Chapman and Hall. New York 2nd ed. Pp: 288.

Hussein,R.A (2016).Evaluation Antioxidant and Antibacterial Activities of n-Butanol Fraction of Conocarpus Erectus L. Leaves Extract. Int. J. Pharm. Med. Res. 2016; 4(6):394-400 ISSN: 2347-7008 International Journal of Pharmaceutical and Medicinal Research Journal homepage: www.ijpmr.org Research Article

Jaffer, H. J.; M.J. Mohmood; A.M. Juwad ; A. Naji, and A. AL-Naib.1983. Phytochemical and biological screening of spme Iraqi plants. Fitoterapia, Lix.299.

Klaus, T., Joerger R., Olsson E. and Granqvist C. G. (1999). Silver-based crystalline nanoparticles, microbially fabricated. J Proc Natl Acad Sci. USA. 96: 13611-13614.

Kumar A, Yadav N, Bhatt M, Mishra N, Chaudhary P and Singh R 2015 Sol–gel derived nanomaterials and it’s applications: a review Res. J. Chem. Sci. 5 98–105

Liogier, H.A. (1990).”Plantas medicinales de Puerto Rico y del Caribe. Iberoamericana de Ediciones”, Inc, San Juan J., Pp (1-566).

Mradu, G ; S. Saumyakanti; M. Sohini; and M. Arup.2012. HPLC Profiles of Standard Phenolic Compounds Present in Medicinal Plants. International Journal of Pharmacognosy and Phytochemical Research, 4(3):162-167.

Nair, B and Pradeep T. (2002). Coalescence of nanoclusters and formation of submicron crystallites assisted by Lactobacillus strains. Cryst Growth Des. 2: 293-298.

Nasrollahzadeh, M. and S. M. Sajadi. (2015). Green synthesis of copper nanoparticles using Ginkgo biloba L. leaf extract and their catalytic activity for the Huisgen [3 + 2] cycloaddition of azides and alkynes at room temperature. J. Colloid Interface Sci. 457: 141-147

Oliveira, M. M.; D. Ugarte; D. Zanchet and A.J. Zarbin. 2005. Influence of synthetic parameters on the size, structure, and stability of dodecanethiol- stabilized silver nanoparticles. Journal of colloid and interface science, 292(2), 429-435.

Percival, M (1997).”Phytonutrients and detoxification,” Clinical Nutrition Insights, vol. 5, pp. 1–4, 1997.

Pileni, M. P. (2000). Fabrication and physical properties of self-organized silver nanocrystals. Pure Appl Chem. 72: 53-65.

Raheema,R.H (2016). Effect of Pomegranate Peel Extract on some Biochemical and Histopathological Parameters in Experimental Induced Mice with Staphylococcus aureus. Journal of Animal Health and Production. 4(2), 43.

Rajeshkumar S, Venkat Kumar S, Ramaiah A, Agarwal H, Lakshmi T and Roopan S M 2018 Biosynthesis of zinc oxide nanoparticles using Mangifera indica leaves and evaluation of their antioxidant and cytotoxic properties in lung cancer (A549) cells Enzyme Microb. Technol. 117, 91–95.

Rasheeda,F Zafara,Z, Zulfiqar Ali Waseema, Muhammad Rafayb, Muhammad Abdullahc, Mir Md Abdus Salamd, Muhammad Mohsind, and Waseem Razzaq Khane.2019.Phytoaccumulation of Zn, Pb, and Cd in Conocarpus lancifolius irrigated with wastewater: does physiological response influence heavy metal uptake. International Journal of Phytoremediation. https://doi.org/10.1080/15226514.2019.1658711.

Ribereau-Gayon,P.1972.Plant phenols.Oliver and Boyd.USA.Pp: 254.

Rice-Evans, C. (2004). Flavonoids and Isoflavones: absorption, metabolism and bioactivity. Free Rad. Biol. 36: 827-828.

Saad,*, Muhammad Asad Saeed1, Farheen Ansari2, Nureen Zahra2, Zeeshan Masood1, Maria Fareed2, Ayesha Javed (2014). Antibacterial and antifungal activity of conocarpus lancifolius engl. (combreraceae). J App Pharm Vol. 6; Issue 2: 153-155

Saadullah,M. Bashir Ahmad Chaudary, Muhammad Uzair and Khurram Afzal (2014). Antidiabetic potential of Conocarpus lancifolius. Bangladesh J Pharmacol, 9: 244-249

Saleh,G.M, Najim,S.S (2020).Antibacterial Activity of Silver Nanoparticles Synthesized from Plant Latex. Iraqi Journal of Science, 2020, Vol. 61, No. 7, pp: 1579-1588 DOI: 10.24996/ijs.2020.61.7.5

SAS.2010.SAS/STAT Users Guide for Personal Computer. Release 9.13.SAS Institute, Inc., Cary, N.C., USA.

Shihata, I. M. (1951). A pharmacological study of Anagallis arvensis.M.D. Vet. the thesis,Cairo University.

Sibanda, T., and Okoh, A. I. (2007). The challenges of overcoming antibiotic resistance: plant extracts as potential sources of antimicrobial and resistance modifying agents. Afr. J. Biotechnol. 6, 2886–2896.

Suleman, P., Redha, A., Afzal, M. & Al-Hasan, R., Temperature-induced Changes of Malondialdehyde, Heat-Shock Proteins in Relation to Chlorophyll Fluorescence and Photosynthesis in Conocarpus Lancifolius (Engl.), Acta Physiologiae Plantarum, 35(4), pp. 1223-1231, 2013.

Tenover, F. C (2006). “Mechanisms of antimicrobial resistance in bacteria,” The American Journal of Medicine, vol. 119, no. 6, pp. S3–S10, 2006.View at: Publisher Site | Google Scholar.

Veerasamy, R.; T. Z. Xina; S. Gunasagaran; T. F. W. Xiang; E.F.C. Yang; N. Jeyakumar, and S. A. Dhanaraj.2011. Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities. Journal of Saudi Chemical Society Volume 15, Issue 2, 113-120.

Vidya, C., S. Hirematha, M. N. Chandraprabhab, M. A. L. Antonyraja, I. V. Gopala, A. Jaina and K. Bansal. (2013). Green synthesis of ZnO nanoparticles by Calotropis Gigantea. Proc. of Nat. Conf. on Women in Sci. & Eng. (NCWSE 2013), SDMCET Dharwad. Int. J. C. Eng. Technol. 118-123

Willner I, Baron R. and Willner B. (2006). Growing metal nanoparticles by enzymes. J Adv Mater. 18: 1109- 1120.

Zheng-Mu, M.; Sakai, I.; Ose, Y.; Sato, T.; Nagase, H.; Kito, H.; Mizuo, M.;One, K. and Nakane, H (1990). Antimutagenic activity by medical plants in trailonal Chinese medicines. Shoy. Akugaku. Zasshi, 44(3)225-229.