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Analysis of active ingredients of Conocarpus erectus on pseudomonas aeruginosa

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Pseudomonas aeruginosa, which causes recurrent infections, is one of the predominant pathogens in cystic fibrosis (CF) patients. A large variety of cell-associated and extracellular virulence factors that are involved in P. aeruginosa's pathogenesis and contribute to its pathogenesis provide this pathogen tremendous versatility. In this research, A GCmas assay are performed, quercetin is manufactured and measure the effect of different concentration of Conocarpous eractus extract and quercetin on pseudomonas spp. Biofilm formation found that the first pseudomonas spp. isolate is more sensitive to antibiotics, so it is more sensitive to the plant extract, so it is the best result. Unlike the last isolate, where it is the strongest antibiotic resistant, so the effect of the extract is less severe, but it inhibited the biofilm.

Keywords: Pseudomonas aeruginosa; Conocarpous; Cystic fibrosis (CF); GCmas assay.

1. INTRODUCTION

The World Health Organization (WHO) defined medical plants as any plant that contains parts that can be used for beneficial purposes or that are precursors to the semi-synthesis of chemopharmaceuticals [1]. About 2/3 of the population still uses plants as traditional medicines to protect their health from illnesses [2]. Conocarpous is a dicot that is branching, multi-evergreen, and. Plant extracts have been demonstrated to be effective inhibitors of trypanosome, leishmania, and plasmodia [3, 4]. A traditional medicine for anemia, conjunctivitis, diabetes, diarrhea, headaches, gonorrhea, bleeding, tumors, syphilis, and fever is conocarpous in several regions. The plant extracts' broad-spectrum antibacterial activity supports its usage as a health treatment in traditional medicine [1].

Conocarpus also has a variety of antibacterial and antifungal activities against a number of pathogenic pathogens, includes Klebsella pneumonia, Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, and Staphylococcus aureus [5,6].

Nanoparticles NPs are particulate that have at least one dimension less than (D1) [7]. Nanoparticle NPs are recently explored in many studies and it is used in the manufacture of many medical and non-medical industries, used as an anti-fungal and anti-bacterial, and hence, at [1] used AgNPs Conocarpous erectus leaves aqueous extract are tested against some breast cancer cell lines. Nanoscience and nanotechnology are inherently transdisciplinary fields of science. With new bio-based approaches, there is a need for biologists to understand not only the basic principles of nanoscience, but also the technologies and methods traditionally employed to characterize nanomaterials. We hope that this review can help to inspire new collaborations across different scientific disciplines, by helping biologists to identify the best technologies and partners to characterize their nanomaterials. At the same time, we recommend to take potential biological risks of these new materials into careful consideration already during the planning phase of such experiments [8].

According to research, the water-soluble polyphenols known as tannins, which can be used as dyes, can be found in the leaves and bark of Conocarpus species. The entire plant of C. erectus has been found to contain quercetin-3-Oglucoside, apigenin, catechin, catechin-3-O-glucoside, rutin, quercetin, and kaemferol-3-O-glucoside The -6-O-gallic acid [5]. Depending on the plant section, C. erectus methanol extracts have varying amounts of phenolic content. Its leaves, flowers, stems, and fruits, respectively, have 581.1 mg/g, 433.9 mg/g, 236.8 mg/g, and 216.1 mg/g of caffeine [9].

Molinspiration, a program that can assess a molecule and provide a variety of metrics, is used in silico research. It has the ability to determine a compound's potential to act on particular pharmacological targets. The preferred orientation can be used to forecast the degree of binding affinity or link between two molecules. Some well-known docking strategies maintain the protein conformation's rigidity while treating the ligand as flexible [10]. The scope of this work is to analyze components of Conocarpus plant and study their effectiveness on Pseudomonas bacteria, as will be proven in this research.

2. MATERIALS AND METHODS

2.1 leaves of the plant collection

leaves of plant are collected from Baghdad city/Iraq, then ished with running tap water to get rid of the dust and then dried by the heat 40- 45˚C for one day, then leaves are grinded by blender machine [11].

2.2 Ethyl acetate extraction (flavonoids)

The extraction is done in accordance with [12]. 100 g of the dried conocarpous leaves are added to 3 L of D.W. and heated to 70°C for 30 minutes. Filter paper is used to filter the extract, and 1.5 L of ethyl acetate is used to extract the supernatant three times. To separate flavonoids, the organic phases are extracted three more times with 1.5 L of an aqueous citric acid solution. The citric acid solution had a 10 mg/L concentration. The solid left over after evaporating the aqueous extract is weighed in accordance with the following [13] equation:

% flavonoids (w/w) = (weight of extract of flavonoids/weight of plant sample) 100

2.3 Quercetin preparation

Conocarpous extract (0.2 mL) is centrifuged (3 mins, 15,000 rpm) to clear out the solid object. The sample is then purified using solidphase extraction and protein precipitation (Sep-Pak, 200 mg C18 Cartridge, Water, USA). In a nutshell, methanol is used to activate the cartridge and distilled water is used to condition it. After loading the sample through the cartridge, it is desalted with distilled water. Finally, 60% methanol is used to elute the analytics [25-29].

2.4 GC-Mass Analysis

This examination is conducted on alcoholic extracts that used to indicate the compound Conocapous leaves, the methanol extract that dissolved in DMSO and filtered by syringe Millipore (μM 0.45) that the filtrate submitted to the GC-Mass spectrometry which carried out by gas chromatography-mass-spectrometry, for the determination of negative ions(m/z) through using column. SS, 30m 0.250 m I.D. x 0.25 m, 5% phenyl methyl Sillox(1629.5), HP-5MS. The parameters are then applied [2].

2.5 Microorganisms

The Pseudomonas spp. bacterial strains are acquired from the University of Baghdad's Biology Department's labs. Afterwards, the germs are clinically isolated from afflicted patients who are hospitalized. The cultures are incubated for 24 hours at 37 degrees Celsius after being subcultured onto cetrimide agar plates. After incubation, Single colonies are transferred from the plates and injected into the brain heart broth-filled tubes. The cultures are cultured at 37°C for 24 hours before to use [14].

2.6 Effect on adherence and biofilm formation

The impact of various dosages of plant extracts on adhesion and biofilm-forming capacities is investigated using polystyrene flat-bottomed microtitre plates, with certain modifications, as described by [15]. 1% (w/v) glucose is added to trypton soya broth that had been diluted with overnight cultures (1:100). A 96-well polystyrene microliter plate is used for the transfer of the culture, which is then incubated at 37°C overnight. After incubation, the plate is gently rinsed twice with normal saline, the supernatants from each well are removed, and the plate is dried and fixed for an hour at 65°C. In order to undertake the quantitative examination of the biofilm, 200 l of 95% ethanol is added to all the plates, which are then gently rinsed after being stained with 0.1% (w/v) crystal violet for 10 min. The biofilm is lastly assessed at 630 nm using a microplate reader while the de-staining solution included methylene blue [16].

2.7 Computer-aided studies and theoretical research

In order to obtain a complete picture of the created molecules 3a-t, an in silico study is carried out. The physicochemical, pharmacokinetic, and toxicological characteristics of compounds are calculated using Molinspiration, and drug-likeness and bioactivity analyses of leads are predicted using Molinspiration cheminformatics software [17]. Molinspiration assists in the calculation of crucial molecular properties like polar surface area and the number of hydrogen bond donors and recipients in addition to forecasting the bioactivity scores for the most important drug targets (GPCR ligands, kinase inhibitors, ion channel modulators, enzymes, and nuclear receptors) [16].

3. RESULT AND DISCUSSION

3.1 GC Mass

The GC-MS analysis revealed to the crude extract of methanol Conocarpous leaf extract. The results showed several compounds with the potential of antimicrobial and antioxidant activity such as alkaloids, phenols, flavonoids and terpenoids are identified. GC-MS analysis of the Conocarpous crude methanolic extract as showed at (figure 1) that the extract contains the following compounds: benzo [h]quinolone C13H9N (alkaloid) ; 2,4-dimethyl C7H14; benzene C6H6 ; and caryophyllene oxid (phenol) C15H24O with molecular weight(m. w.) 179.22; 98.19; 78.11; and 220.35 successively. It thought that tested microorganisms are inhibited due to the quinolones in the extract.
At GC-MS results for Conocapous crude extract of [2], and [19] found benzo [h]quinolone; 2,4-dimethyl; benzene; and other compounds similar to the compounds find at this study.

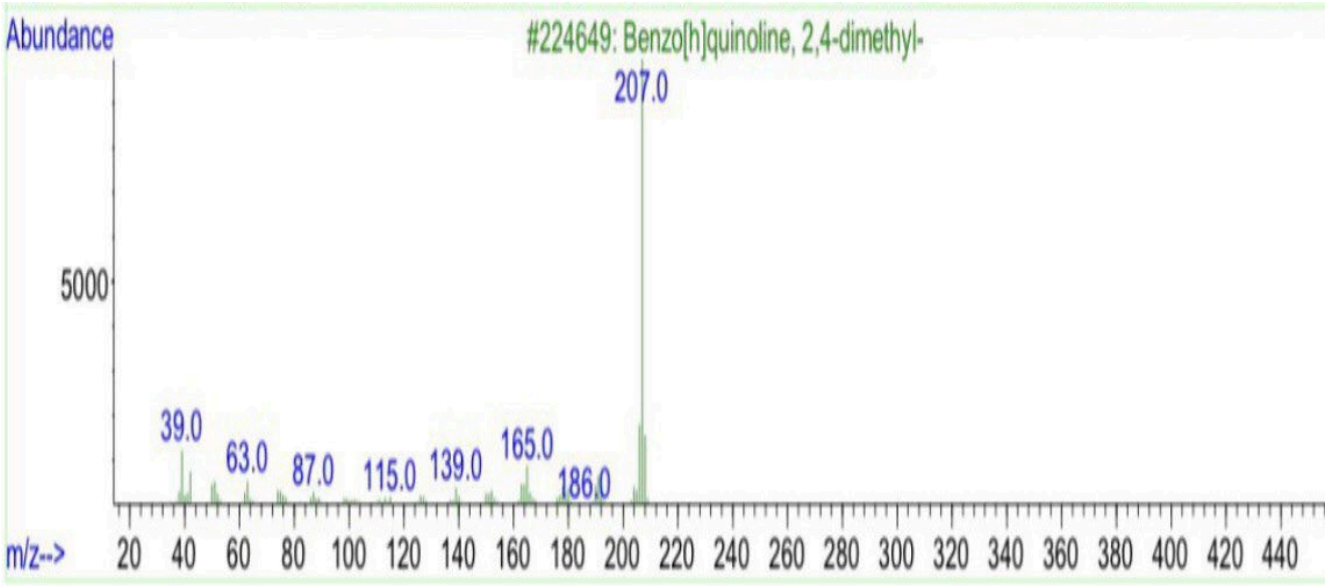

Figure 1 The Compounds of methanolic extract produced by the dried Conocarpus leaves detected by GC-MS.

3.2 Theoretical study

Theoretical Biological Activities depending on (Molinspiration bioactivity score) (Molinspiration Home Page, 2017). The results shown in Table 1 must range from the worst -2 to the best 2.

Table 1 The theoretical Biological Activities of Quercetin by molinspiration data.

Receptor	Quercetin
GPCR ligand	0.06-
Ion channel modulator	0.19-
Kinase inhibitor	0.28
Nuclear receptor ligand	0.36
Protease inhibitor	0.25-
Enzyme inhibitor	0.28

Based on result of Table 1, quercetin have kinase, enzyme inhibitor and nuclear receptor ligand effect, which mean it has anti- cancer activity by the suppression of a significant number of kinases and being a polyphenol formed from plants, quercetin has several free hydroxyl groups, making it simple for various enzymes to break it down. The majority of studies [20,21, 22] have concentrated on quercetin's antioxidant characteristics, impacts on various enzyme systems, and effects on biological pathways involved in carcinogenesis.

3.3 Bacterial susceptibility to different kinds of antibiotics

Isolated bacteria are more resilient to certain antimicrobial remedies. Table 2 summarizes the results of the disc diffusion method used to test Pseudomonas isolates for susceptibility to seven different antibiotics [23]. These antibiotics are utilized in this experiment because their mode of action prevents the production of cell walls.

Table 2 Bacterial susceptibility to antibiotics. R: Resistant, I: Intermediate, S: Sensitive, CIP: ciprofloxacin, AK: amikacin, IPM: imipenem, AX: Amoxicillin.

Sample code	CIP	AK	IPM	AX
ISO 1	S	S	I	S
ISO 2	S	I	I	S
ISO 3	I	S	R	R
ISO 4	I	R	R	R
ISO 5	R	R	R	R

3.4 Effect of different concentration of Conocarpous eractus extract and quercetin on pseudomonas spp. Biofilm formation.

The result at figures (2,3,4,5,6) show that the first isolate is more sensitive to antibiotics, so it is more sensitive to the plant extract, so it is the best result. Unlike the last isolate, where it is the strongest antibiotic resistant, so the effect of the extract is less severe, but it inhibited the biofilm. [24] find that methanolic extract of Conocarpus erectus leaves reduced the biofilm formation of P. aeruginosa at 6.25 mg/ml, and inhibited 80% and 100% of the biofilm formation at 25 and 50 mg/ml respectively. at [24] study, P. aeruginosa show multi-drug resistant to common antibiotics used.

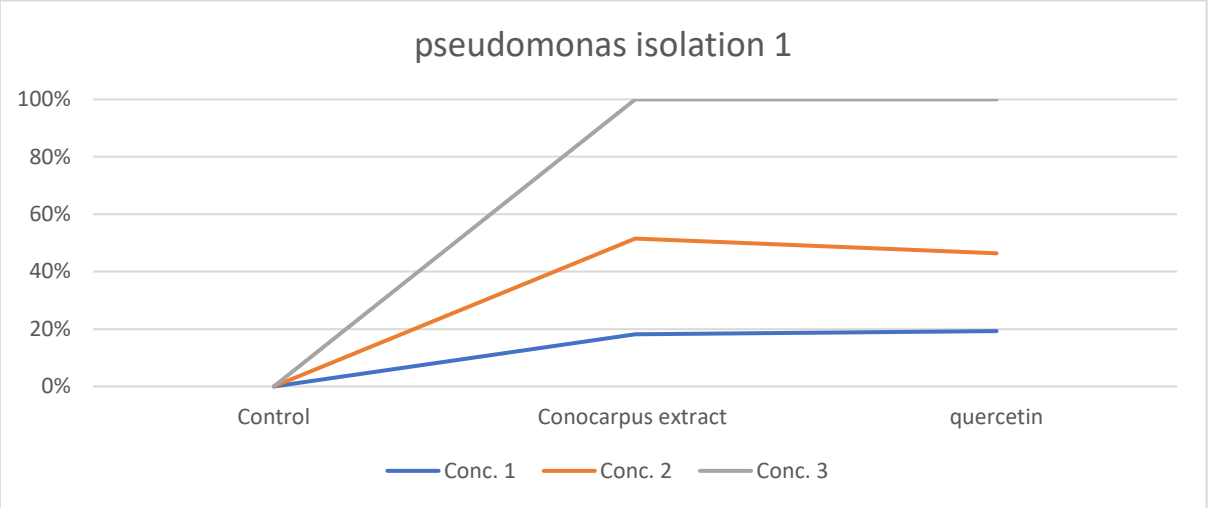

Figure 2 showed the effect of (Conocarpus extract and quercetin with different concentrations 50, 75 and 100 mg/ml) on Pseudomonas isolation 1. A UV-VIS spectrophotometer identified the biofilm inhibition and displayed it as a percentage of inhibition (with respect to untreated control). Data are shown as the mean standard deviation of three independent experiments.

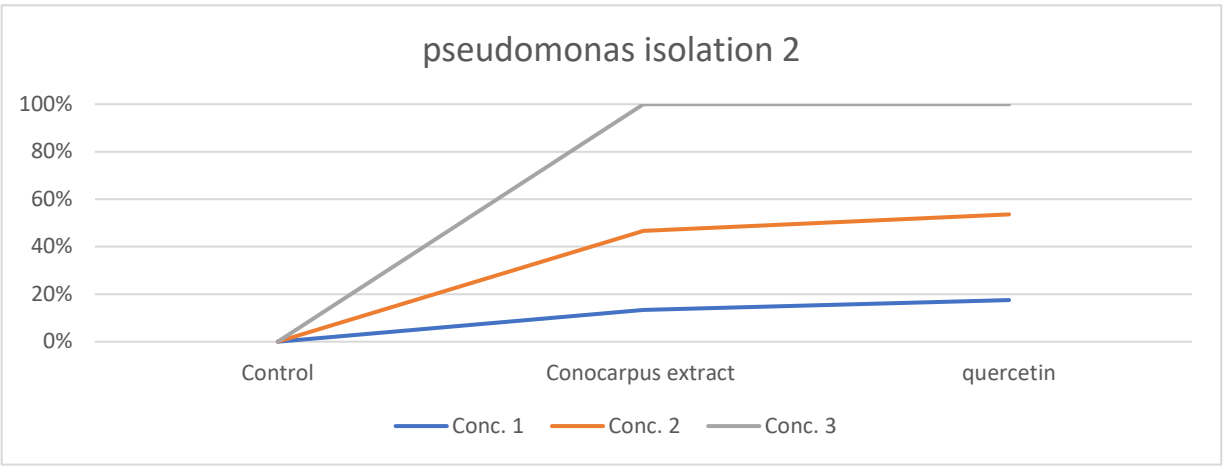

Figure 3 showed the effect of (Conocarpus extract and quercetin with different concentrations 50, 75 and 100 mg/ml) on Pseudomonas isolation 2. A UV-VIS spectrophotometer identified the biofilm inhibition and displayed it as a percentage of inhibition (with respect to untreated control). Data are shown as the mean standard deviation of three independent experiments.

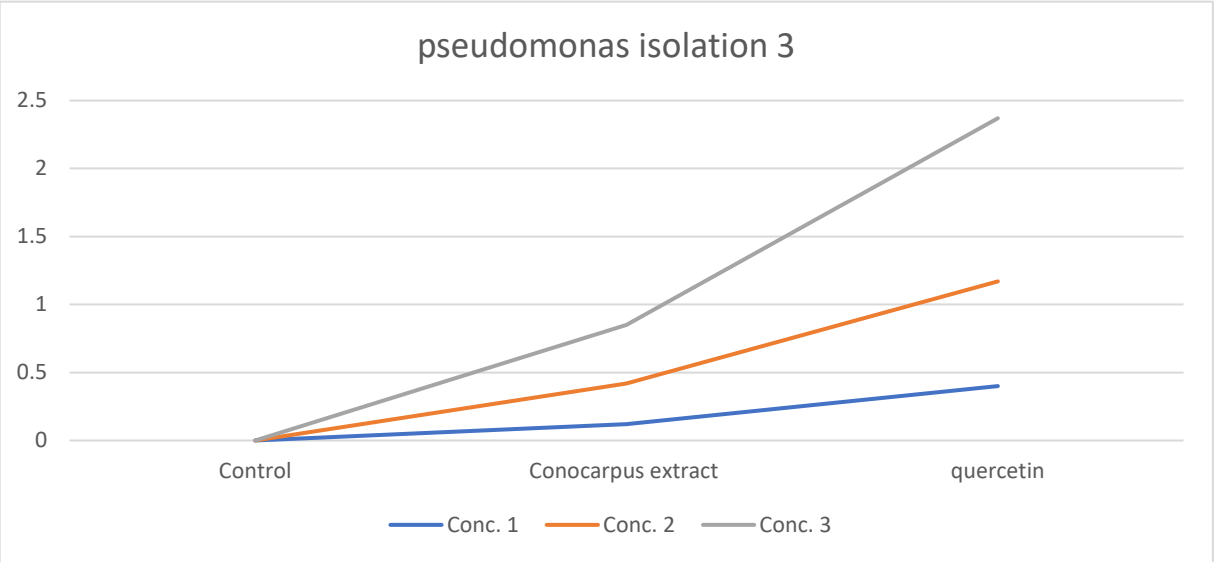

Figure 4 showed the effect of (Conocarpus extract and quercetin with different concentrations 50, 75 and 100 mg/ml) on Pseudomonas isolation 3. A UV-VIS spectrophotometer identified the biofilm inhibition and displayed it as a percentage of inhibition (with respect to untreated control). Data are shown as the mean standard deviation of three independent experiments.

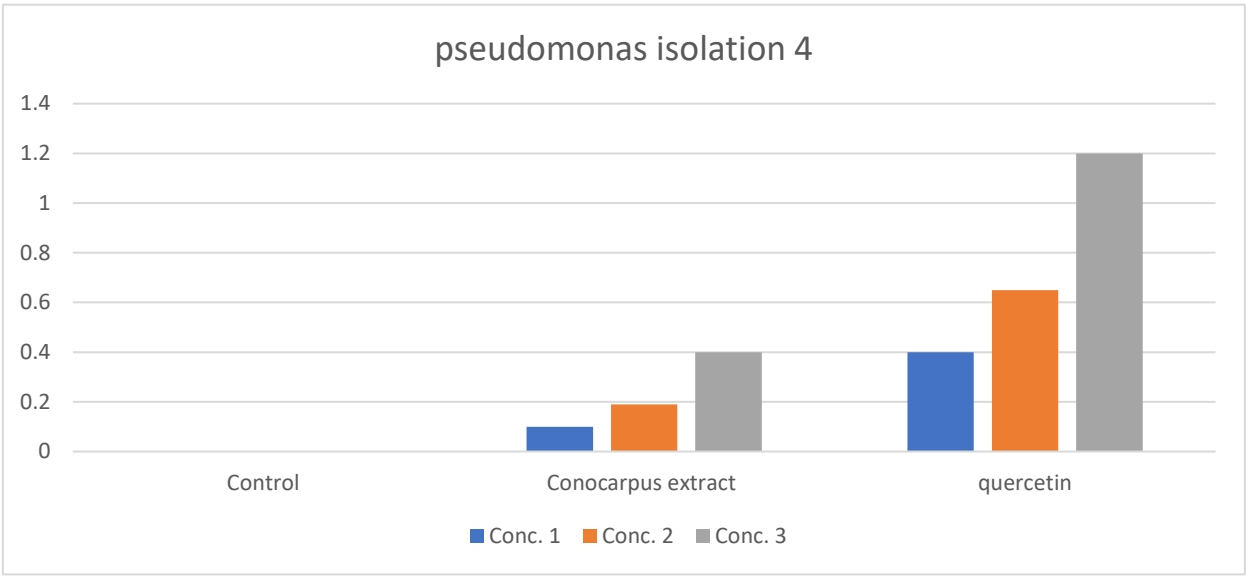

Figure 5 showed the effect of (Conocarpus extract and quercetin with different concentrations 50, 75 and 100 mg/ml) on Pseudomonas isolation 4. A UV-VIS spectrophotometer identified the biofilm inhibition and displayed it as a percentage of inhibition (with respect to untreated control). Data are shown as the mean standard deviation of three independent experiments.

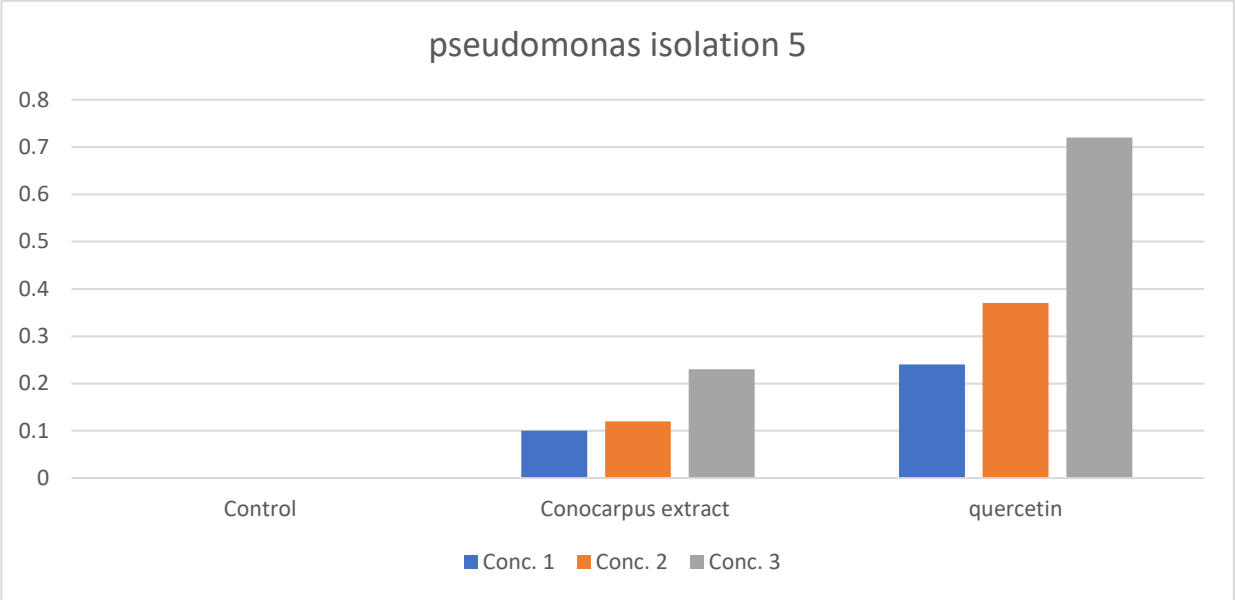

Figure 6 showed the effect of (Conocarpus extract and quercetin with different concentrations 50, 75 and 100 mg/ml) on Pseudomonas isolation 5. A UV-VIS spectrophotometer identified the biofilm inhibition and displayed it as a percentage of inhibition (with respect to untreated control). Data are shown as the mean standard deviation of three independent experiments.

4. CONCLUSIONS

The quercetin derivatives were successfully created from Conocarpus leaves. According to the research's conclusions, quercetin derivatives were given after the data were analyzed using Molinspiration, were it more potent with Kinase, Enzyme Inhibitor and Nuclear receptors, which mean quercetin can easily work as anti-cancer, anti-microbial, destroy fatty acid and lipophilic acid and prevent biofilm formation by various bacteria species.

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