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*Original article*

# Anti-Cancer, Anti-Inflammatory, Antibacterial, Antifungal, Anti-Oxidant and Phytochemical Investigation of Flowers and Stems of *Anacyclus Clavatus* Plant Extracts

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**Abstract**
The alternative medications have been used in Libya for many years. Libya is considered rich in medicinal plants. Thousands of these plants grow naturally throughout the country, particularly in the Al-Jabal Al-Akhtar region. This study aims to estimate the antioxidant activity, phytochemical screening, total phenols contents, antibacterial, anti-inflammatory, and anti-cancer investigations of the flowers and steams of *Anacyclus Clavatus* extracts. Two different extracts were applied in this study (Aqueous and Alcohol). The results showed the presence of some active natural compounds as carbohydrates and/or glycosides, sterols and/or triterpenes, tannins, flavonoids, and cardiac glycosides in all studied plant species with different amounts. The total phenols and antioxidant activity contents were 9.1 and 11.40 ppm for stems and flowers, respectively. On the other side, the contents of antioxidants were 3.26 and 3.93 ppm in stems and flowers of the studied extracts. The antibacterial and antifungal applications showed that the extracts gave inhibition activities on the selected bacteria species of (Staph and Klebsiella) and fungi species of (Aspergilluis and Penicillium). For the anti-inflammatory investigation. The extracts of flowers and stems gave indicating that the compound with less Hemolysis Inhibition % results in good inhibitory activity. The anti-cancer activities of the plant extracts were applied on Cancer cell lines (HePG2). The plant extract was shown to have potential antitumor action against cell lines. The cytotoxicity of the plant extract was tested using human Liver (HepG 2) cell lines.
**Keywords**. Anacyulus Clavatus, Inflammatory, Antibacterial, Anticancer.

## Introduction

Plants typically contain many secondary metabolites, such as phenols, quinones, flavonoids, alkaloids, tannins, sterols, and saponins. These metabolites are essential sources of several pharmaceutical uses. Medicinal plants play a crucial role in pharmacological studies and drug development. Plants have been classified as a rich source of drugs because they produce a wide range of active molecules, most of which likely evolved as chemical defenses against predatory or infectious and antioxidant compounds [1]. Oxidative changes in the metabolic pathway causing disorders are oxidative changes in metabolism causing many diseases [2]. Such bioactive compounds have gained special attention due to their potential protection of the human body versus the oxidative stress caused by many diseases, including cardiovascular disorders, cancer, aging, and antimicrobial properties [3].

Several chronic diseases, including cardiovascular disorders and at least some kinds of cancer, are caused by free radical oxidation of lipids, proteins, and nucleic acids. The majority of herbal plants which probably evolved as a chemical protection against predation or infection and antioxidant compounds [4]. Many antioxidant molecules, including phenolics, carotenoids, anthocyanins, and tocopherols, can be detected in some plants. Approximately 20% of known plants were used in pharmaceutical studies, with positive effects on healthcare. Plants that have beneficial phytochemicals can complement the needs of the human body by serving as natural antioxidants. Many studies have shown many plants to be rich sources of antioxidants as (vitamins A, C, E and phenolic compounds) they all act as plant borne antioxidants, such as flavonoids, tannins and lignins.

It has been reported that the exploration of natural products, including plant extracts, is revolutionizing the field of drug discovery, offering promising avenues for new treatment options [5]. They are considered of importance due to their properties as an expansive source of helpful phytochemicals, which will lead to the development of novel drugs. Most of the phytochemicals from plant sources such as phenols and flavonoids have been detailed to have a positive effect on health and cancer avoidance. Medicinal plants have been utilized since old times to treat illnesses [6].

*Anacyclus clavata* is also known by various names like white anacyclus and white Asteraceae. Northern Africa: Libya, Algeria, Tunisia, and Morocco Macaronesia: Lanzarote Southwestern Europe: Ibiza, France,


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Majorca, Spain, Menorca, Portugal. Southeastern Europe: Croatia, Italy, Greece, Turkey, Malta, Sicily, Sardinia. The study objectives were to measure the antioxidant activity of the plant samples under investigation, identify phytochemical screening of the flowers and steams of *Anacyclus Clavatus* plant growing in the Al-Jabal AL-Akhder region of Libya, and assess the antimicrobial, anti-inflammatory, and anti-cancer efficiency activity.

## Methods

### *Sampling*

The flowers and stems of *Anacyclus Clavatus* plant were chosen for this study. The plant was collected in the spring of 2024 from the Al-Gabel Al-Kadar region. The collected samples were identified in *Seliphium* herbarium, Botany Department, Faculty of Science, Omar Al- Mukhtar University. The plant taxonomy is given in Table 1.

***Table 1. The taxonomy of the studied plant.***

| **Kingdom** | ***Anacyclus Clavatus*** |
|---|---|
| **Clade** | Angiosperms |
| **Family** | Asteraceae |
| **Genus** | Anacyclus |
| **Species** | A. clavatus |

### *Samples preparation*

The Flowers and Stems of the studied plant were separated and washed several times with distilled water. The samples were then dried in a dark and dry place. Then, the samples were ground by mortar and stored in polyethylene bottles until analysis. The extraction was carried out by taken 10 gram of each sample and transported into beaker containing 500 ml of solvent (in this distilling water or Ethyl alcohol), the extraction system was used by optimized the temperature degree at 75 $^0$C for water and 55 $^0$C for alcohol, about two hours, the mixtures were cooled then filtrated and applied immediately for phytochemical screening [7-11].

### *Phytochemical screening*

All the phytochemical screening tests were carried out according to methods described by many studies [12-15].

### *Sterols and/or triterpenes*

Libermann-Burchard's test was used to identify the sterols and/or triterpenes. It involved mixing one milliliter of each sample's chloroform extract with 0.3 milliliters of acetic anhydride, followed by a few drops of strong sulfuric acid placed along the dry test tube's side. When sterols and/or triterpenes are present, the chloroform solution turns green, and the intersection of the two layers produces a reddish-violet color.

### Test for flavonoids

The studied herbal plants' alcohol and aqueous extracts were further extracted using 1% hydrochloric acid. Each extract was then put through the following test: 10 ml of each extract is made alkaline, and if flavonoids are present, a light-yellow color is created.

### *Test for alkaloids*

The extracts of the tested herbal plant were further extracted with 20ml of dilute hydrochloric acid, cooled and rendered alkaline with dilute ammonium hydroxide solution, then extracted with chloroform. The chloroform extract is subjected to the following tests*:*

### *Dragendorff*

The preparation of the reagent*:* Solution (a): About of (0.85 g) of basic bismuth nitrate was dissolved in a mixture of 10 ml acetic acid and 40 ml distilling water. Solution (b): about (8 g) of potassium iodide was dissolved in (20) ml water. Stock solution: Equal volumes of solutions (a) and (b) are mixed. A few drops of chloroform extract were applied to filter paper, allowed to dry, and sprayed with the reagent. An orange colour is observed in cases of the presence of alkaloids.

### *Test for tannins*

The extracts (alcohol and aqueous) of the tested herbal plants were further extracted with ethanol 50%, filtered, and the hydro-alcoholic clear solution subjected to the following test*:*


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***Ferric chloride test***
One ml of the reagent (1% $FeCl_3$) was added to the hydro-alcoholic solution. Blue colour develops in cases of the presence of pyrogallol tannins.

***Test for anthraquinones***
***Bornträger's test***
One ml of each successive extract of the successive extracts of aqueous ammonia or caustic soda is added and shaken. The rose-red colour in the aqueous layer develops as a result of the presence of anthraquinone glycosides.

***Modified-Bornträger's test***
One ml of each extract of the successive extracts of the tested herbal preparations is hydrolyzed with alcoholic potassium hydroxide, the acidified and continues as Bornträger's test. Rose-Red develops in the aqueous layer in cases of the presence of anthraquinones.

***Test for saponins***
Five ml of tape water is added to (1 ml) of each extract, then shaken vigorously for five minutes, froth develop having (1cm) high and persists for (15minutes) indicates the presence of saponin. In this study the phytochemical screening, antioxidant activity, total phenols of the studied samples were expressed as the following codes, Table (2).

***Table 2. The code of samples***

| Sample Code | Sample Type |
|---|---|
| 1 | S1 Stems of |
| 2 | F2 Flower of |

***Determination of total phenols by Folin Ciacalteu Method***
This method was carried out to determine phenolic compounds in the aqueous and Ethanol extracts, where 10 ml was added to 3ml of distilled water with Folin Ciacalteu reagent. According to the method of Slinkard and Singleton, using Gallic acid as a standard. Samples (leafs and seeds of barley plant) were introduced into test curettes, and then 0.5mL of Folin-Ciocalteu reagent and 2 ml of $Na_2CO_3$ (20%) were added. The absorbance of all samples was measured at 650 nm using UV–Vis spectrophotometer after incubating at (1 min) and cooled for (15 min). Results were expressed as milligrams of Gallic acid equivalent per gram of fresh weight [16].

***Determination of antioxidant capacity by Prussian blue method***
"First, one gram of the sample powder was treated with petroleum ether to remove fats. The remaining material was then extracted in two steps: it was stirred with 10 ml of methanol twice, followed by stirring with 10 ml of a 1% hydrochloric acid-methanol mixture (v/v). The combined extracts were concentrated by removing the solvent under a vacuum. The remaining solid was then dissolved in methanol. A small portion of this solution was diluted with water and then mixed with several chemicals, including potassium ferricyanide, hydrochloric acid, and ferric chloride. This mixture was allowed to react for a few minutes, resulting in a blue color. The intensity of this color was measured at a specific wavelength (720 nm) to obtain a quantitative analysis, compared to a reference sample (blank) [17-20].

***Antibacterial and antifungal Studies***
The bacteria used in this study were isolated from Al Bayda Central Hospital and grown on selective environments to identify them by (Turbidimetric Method). The optical density (OD) of the assigned broth culture was measured using a spectrophotometer set to a wavelength of 600 nanometers. To improve accuracy, the culture was slightly diluted before taking the readings. These measurements were repeated throughout the experiment at chosen time points. The nutrient broth medium was prepared according to the company's instructions by dissolving 13 grams of the medium in 950 ml of distilled water, adjusting the pH to 7.2. Then increasing the volume to 1000 ml and pH using an autoclave at a temperature of 121°C for 20 minutes and used to activate the isolates [21]. Add 100 ml of fresh culture medium and incubate for 24 hours. A spectrophotometer was used to estimate the turbidity values.

***Anti-inflammatory Studies***
***Preparation of erythrocyte suspension***
Blood from healthy volunteers (3 ml) was collected in tubes containing heparin and spun at 3,000 rpm for 10 minutes to separate the red blood cells. The cells were then washed with saline and adjusted to a 40% concentration using a special salt solution (phosphate buffer at pH 7.4) that mimics the body's fluids. This


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solution was made by dissolving specific amounts of sodium phosphate salts and sodium chloride in water. The prepared red blood cell suspension was then ready for use in experiments.

***Hypotonicity induced hemolysis***

To assess the extract's effects, researchers prepared solutions in varying concentrations (100-1000 µg/ml) using both distilled water (hypotonic) and a balanced salt solution (isotonic). These solutions were then placed in centrifuge tubes, two per concentration level. For comparison, additional tubes contained either just the solvent (distilled water) or a standard reference solution (200 µg/ml indomethacin). To assess the effect of an extract on red blood cell lysis, red blood cell suspensions (0.1 mL) were incubated with the extract for 1 hour at room temperature (37°C). Hemolysis was then measured by quantifying hemoglobin release at 540 nm using a spectrophotometer. Complete hemolysis by distilled water served as a positive control (100% hemolysis). The percentage inhibition of hemolysis by the extract was subsequently calculated.

$$\% \text{ Inhibition of hemolysis} = 1 - [(OD_2 - OD_1)/(OD_3 - OD_1))] * 100$$

Where $OD_1$ = absorbance of test sample in isotonic solution, $OD_2$ = absorbance of test sample in hypotonic solution, $OD_3$ = absorbance of control sample in hypotonic solution.

***Anti-cancer Studies***

***Determination of sample cytotoxicity on cells (MTT protocol)***

The MTT was carried out as following steps

The 96-well plate was seeded with a specific concentration of cells (100,000 cells per milliliter, 100 microliters per well). After incubation at 37°C for 24 hours, the cells grew and multiplied to form a complete and continuous layer across the bottom surface of each well. Following the formation of a confluent cell monolayer in 96-well microtiter plates, the culture medium was discarded, and the cells were subjected to two washes with washing medium.

A serial dilution of the test sample was prepared in RPMI medium supplemented with 2% serum (maintenance medium) to create three concentrations with a two-fold difference between each. Small amounts (0.1 milliliters) of each dilution were placed in separate wells. Three wells were left untreated, containing only a nutrient solution to serve as a comparison. The plate was incubated at body temperature (37°C) to allow cell growth. Afterwards, the cells were inspected under a microscope to assess their health for any signs of damage, such as a disrupted cell layer, changes in cell shape (rounding or shrinking), or the presence of granular material within the cells.

MTT solution was prepared (5mg/ml in PBS) (Bio Basic Canada INC). 20 µL of MTT solution were dispensed into each well. To ensure complete mixing of the MTT solution with the culture medium, the plate was then incubated on a shaking table at 150 rpm for 5 minutes. Cells were maintained in a controlled environment (37°C, 5% $CO_2$) for 4 hours to enable the MTT conversion process. Pour out the media. Wipe the plate with paper towels to remove any remaining residue. Disperse the formazan (MTT metabolic product) back into solution by adding 200 microliters (uL) of DMSO. Shake the mixture on a shaking table at 150 rpm for 5 minutes to ensure complete resuspension of the formazan in the solvent. Read the OD at 560nm and subtract background at 620 nm. OD should be directly correlated with cell quantity [22, 23].

***Morphological assay***

The relationship between large-scale changes in cell morphology and the cytoskeleton can be used as an indicator of cell viability. A significant decrease in cell volume is indicative of cellular damage. This decrease is a consequence of protein and intracellular ion loss, which occurs due to a change in the permeability of the cell membrane to sodium and potassium ions.

Necrotic cells: nuclear swelling, chromatin flocculation, loss of nuclear basophilia

Apoptotic cells: cell shrinkage, nuclear condensation, and fragmentation

## Results

***Phytochemical Screening***

The tests of phytochemical screening are depended up on the color observation qualitative tests). Each extract of the studied plants was screened for the following constituents: carbohydrates and/or glycosides, tannins, flavonoids, sterols and/or triterpenes, saponins, and anthraquinone. The obtained results were recorded in Table 3. The results revealed the presence of carbohydrates and/or glycosides, sterols and/or triterpenes, tannins, saponin, flavonoids alkaloids, anthraquinone, and cardiac glycosides were recorded in all studied plants species with presence different contents (Table 3).


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***Table 3. Phytochemical screening of Flowers and stems of studied plant.***

| Phytochemical screening test | S1 AL | F1w | F1 Al |
|---|---|---|---|
| Tannin | +++ | +++ | +++ |
| Flavonoids | ++ | + | ++ |
| Alkaloids | - | - | - |
| triterpines | +++ | + | +++ |
| Saponins | - | + | - |
| Anthraquinine | - | - | - |

*S1AL: Alcoholic Extract of Anacyclus Clavatus stems, F1W: Aqueous extract of Anacyclus Clavatus Flowers. F1AL: Alcoholic Extract of Anacyclus Clavatus Flowers. (+) Present, (++) Moderate content, (+++) High content and (-) Absent.*

The sterols and/or triterpenes were detected in all extracts of the studied plant in both alcoholic and aqueous extracts. The results showed no wide variations of sterols contents observed in the both solvents (water and Alcohol) of flowers and stems of both extracts. For the flavonoid compounds, the results showed the presence of Flavonoids in the extracts (aqueous extracts). The contents of Flavonoids showed high contents in all extracts and no variations in their contents of the studied extracts (Table 3). Alkaloids were detected in all the studied extracts, generally, there is a relative increase of their contents in alcohol extracts compared with aqueous extracts. The tannins compounds were detected in all extracts of the studied plant. Generally, tansies showed small contents in most of the studied plants compared with other constituents. The carbohydrates and /or glycosides were detected in all the extracts of both studied plants. The results indicated that the stems of plants contain high contents of carbohydrate and /or Glycosides compared with the other parts of studied plants. The anthraquinones compound groups were detected in water extracts of studied plants, on the other side the Anthraquinone and saponin compounds not detected in the extracts of alcoholic extracts of all the studied extracts (Table 3). It was stated that the phytochemical analysis of plants depends on the type of chemical compounds and the solvents used during extraction, where the most effective factor on analysis is the polarity of the solvent, which mainly extracts the same compounds of their polarities [8-10].

***Total phenols and antioxidant activity***

The total phenols and antioxidant activity contents were 9.1 and 11.40 ppm for stems and flowers, respectively. On the other side, the contents of antioxidants were 3.26 and 3.93 in stems and flowers of the studied plant. The findings showed that there was a relative increase in flower samples compared with stems (Table 4).

***Table 4. The contents (ppm) of Total phenols and Anti-oxidant activity***

| Samples<br>Content (ppm) | Stems | Flowers | ± SD |
|---|---|---|---|
| Phenol | 9.71 | 11.40 | 1.195 |
| Antioxidant | 3.26 | 3.93 | 0.473 |

***Biological Studies***

***Antibacterial and anti-fungal investigation***

The turbidity method was used to perform the anti-fungal and anti-bacterial investigation. Two bacterial species (Staph and Klebsiella) and two fungus species (Aspergilluis and Penicillium) were used in this study. Figures 1-6 present the antibacterial and antifungal results. Correlations between interference, bacteria, and fungi treated with various concentrations of aqueous *Anacyclus Clavatus* extract are shown in Figure (1).

The temperature in this experiment was fixed at 37 °C. According to the graph, the quantity of bacterial cells (turbidity indicates an increased number of bacterial cells) significantly dropped from a 75% concentration to a 100% concentration. The graph's data demonstrates a positive correlation between the quantity of bacterial cells and the quality of the turbidity. The findings showed that the aqueous extracts of the *Anacyclus Clavatus* plant had an effect on the aspergillus fungus at all concentrations, while the bacteria that were most resistant to the aqueous extract were Staph staphylococcus, with a minimum inhibitory concentration (MIC) of 50% of the plant extract.

Figure 1 shows bacteria and fungi under treatments with different concentrations of aqueous *Anacyclus Clavatus* stem extract. In this experiment, it decreased significantly from the concentration of 50%. For the type of (*penicillium* sp.) fungi, the concentrations of 50% and 75 % had the same effect on the two types of bacteria studied and showed an increase in turbidity and a decrease in permeability.


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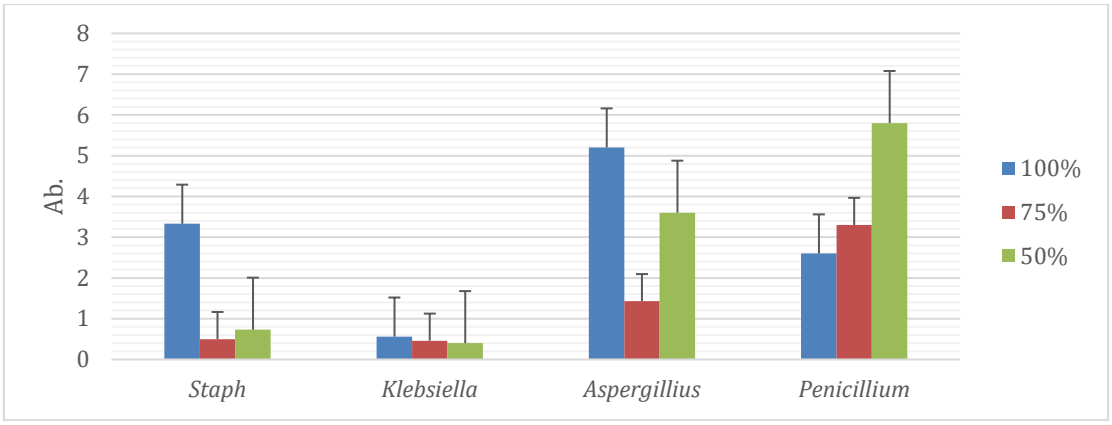


***Figure 1. The effect of aqueous extract of Anacyclus clavatus stem on the studied microbes by measuring turbidity and a MIC account***

Figure 2 shows that the alcoholic extract of the *Anacyclus clavatus* stem appeared; it had a clear effect in inhibiting the growth of all microbes under study, and the most effective concentration was 75%, followed by 50%.

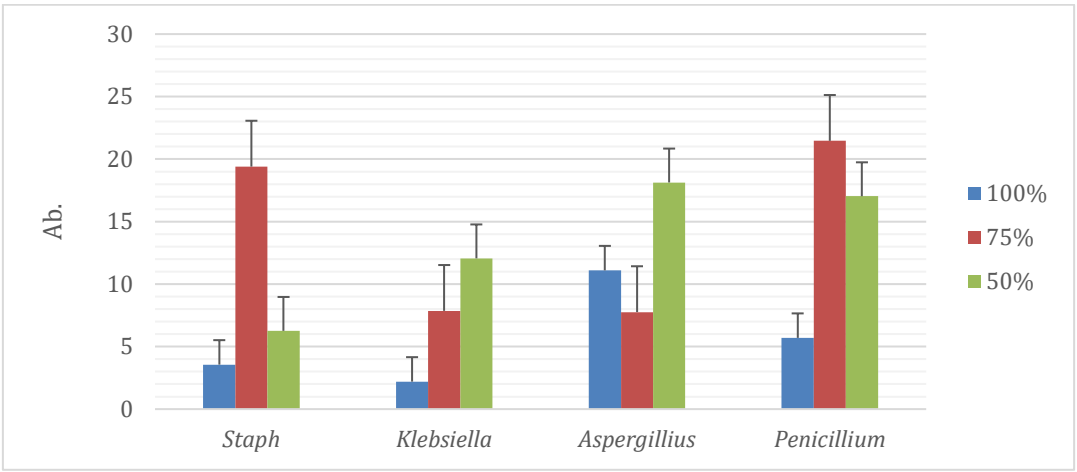


***Figure 2. The effect of alcohol extract of Anacyclus clavatus stem on the studied microbes by measuring turbidity and a MIC account***

Figure (3) shows the different parameters of an aqueous extract *Anacyclus Clavatus* flower showed that the two types of bacteria studied (*Staphylococcus – Klebsiella*) were resistant, and on the contrary, to the two types of fungi (*aspergillus – penicillium*). The lower concentration was more inhibiting of the two fungi.

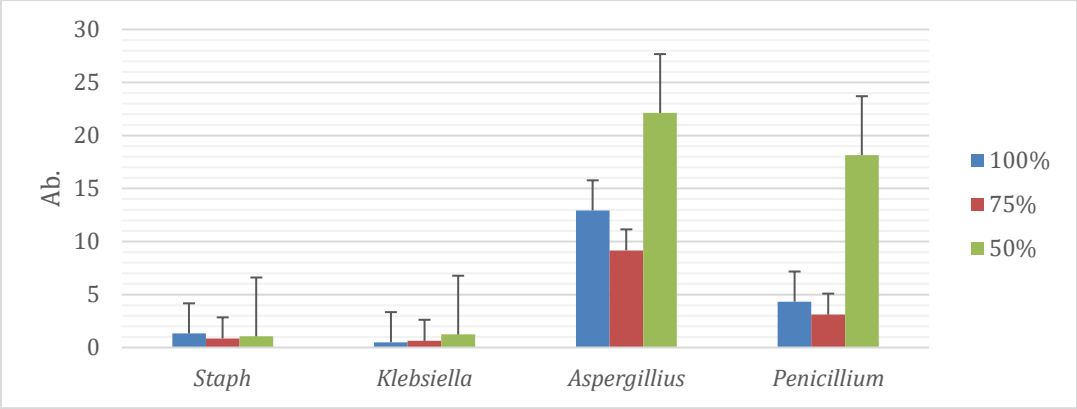


***Figure 3. The effect of extract of Anacyclus Clavatus flower on the studied microbes by measuring turbidity and a MIC account***

Also, the extract the alcohol of the *Anacyclus Clavatus* flowers, concentrations 50 and 75 had a significant effect in inhibiting the growth of the microbes studied. The most resistant organisms to *klebsiella sp.* Bacteria. Figure 4: Showed that the aqueous extract the *Anacyclus clavatus* flowers had a significant effect in inhibiting fungi and bacteria, so it was less effective.


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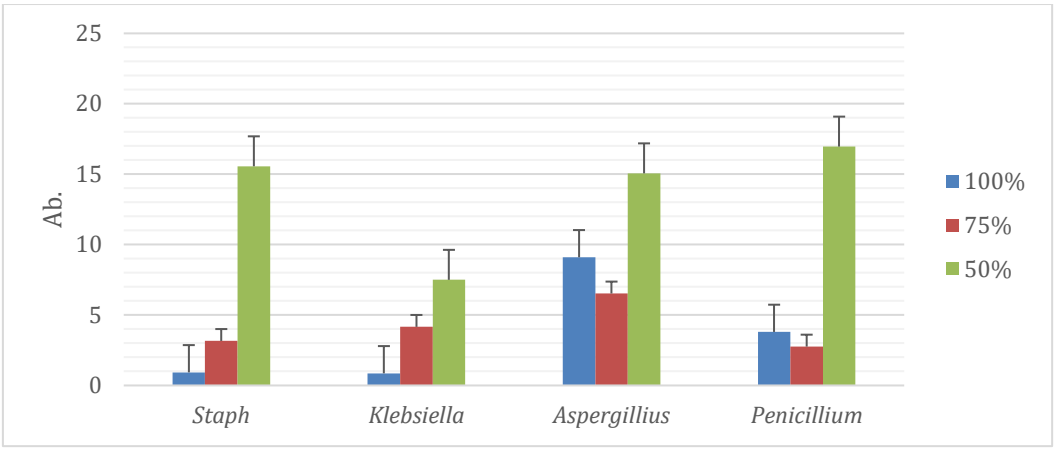


***Figure 4. The effect of alcohol extract of Anacyclus Clavatus flower on the studied microbes by measuring turbidity and a MIC account***

Figure 5 shows that the lowest concentration of the alcoholic extract of the flower of the *Anacyclus clavatus* was very effective in inhibiting all the microbes under study and thus the *Anacyclus clavatus* plant was more effective than the *Anacyclus Clavatus* and gave significant results for all concentrations of the aqueous and alcoholic extracts.

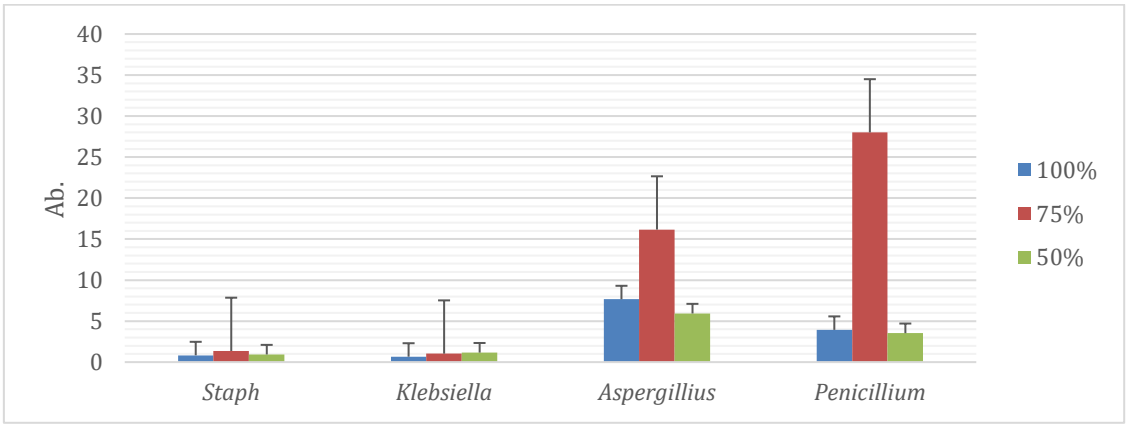


***Figure 5. The effect of aqueous extract of Anacyclus clavatus flower on the studied microbes by measuring turbidity and Mic account***

The effect of alcoholic extract of *Anacyclus clavatus* flower on the studied species of bacteria and Fungi were given in Figure (6), where the high effective was shown at low concentration of 50 % on all selected species.

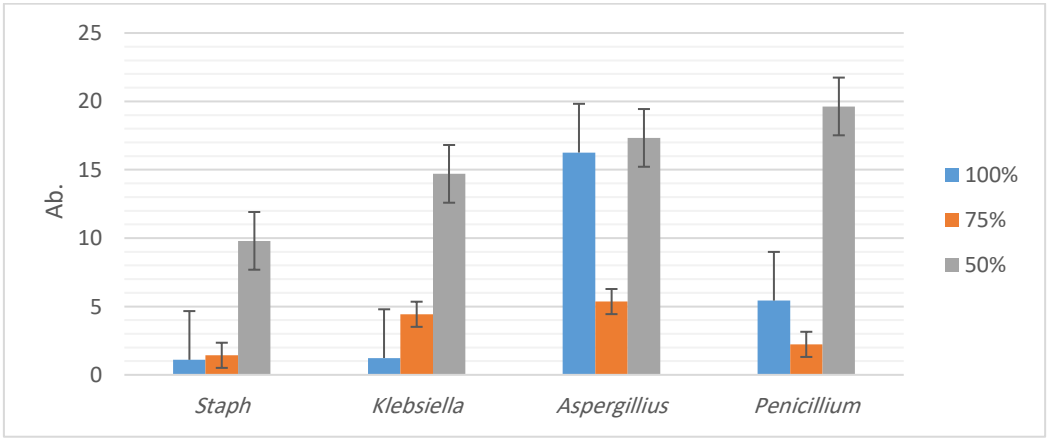


***Figure 6. The effect of alcohol extract of Anacyclus clavatus flower on the studied microbes by measuring turbidity and MIC account***

***Anti-inflammatory studies***

The anti-inflammatory investigation of the studied plant extracts was compared with the standard compound indomethacin, aceclofenac, etoricoxib, and aspirin. The values of Hemolysis % in Tables 5 - 6,


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indicate that the compound with less hemolysis inhibition % results in good inhibitory activity. Among the standard compounds, it responds better to all human red blood cells (HRBC). In this study, the Hemolysis values of the plant extracts were ranged as following (87.60 – 99. %) and (92.2 – 99.90 %), for stems and flowers, respectively. Anti-inflammatory agents primarily target the cyclooxygenase enzyme, which is responsible for converting arachidonic acid into inflammatory mediators known as prostaglandins. The enzyme peroxidase, which forms long membrane channels, converts prostaglandin G2 (PGG2) into prostaglandin H2 (PGH2). The release of chemical mediators and the subsequent opening of these membrane channels trigger the release of arachidonic acid from the membrane, which is then converted into prostaglandins. The extracellular activity of these enzymes is implicated in both acute and chronic inflammatory processes. It was concluded that [24], in vitro anti-inflammatory studies on certain plants by measuring the inhibition of protein denaturation and HRBC membrane stabilization. The % inhibition observed in the current study (43%) is notably higher than that reported in some previous studies [24]. The results of anti-inflammatory investigation were given in Tables of (5 and 6), Table (5) Shows the effect of Sample (aqueous extract of *Anacyclus clavatus* stem) on HRBC hemolytic and membrane stabilization and Table (6) shows the effect of (aqueous extract of *Anacyclus clavatus* flowers) on HRBC hemolytic and membrane stabilization.

***Table 5. Effect of extract Anacyclus clavatus stem on HRBC hemolytic and membrane stabilization***

| Conc. ug/ml | OD R1 | OD R2 | OD R3 | Hypotonic Ab. Mean | Sample with Isotonic solution Ab. | Hemolysis Inhibition % | SD | SE |
|---|---|---|---|---|---|---|---|---|
| Control | 1.115 | 1.112 | 1.118 | 1.115 | 0 | 0 | 0.003 | 0.001 |
| 1000 | 0.099 | 0.101 | 0.103 | 0.101 | 0.096 | 99.5 | 0.002 | 0.001 |
| 800 | 0.114 | 0.112 | 0.119 | 0.115 | 0.084 | 97.6 | 0.004 | 0.001 |
| 600 | 0.131 | 0.133 | 0.136 | 0.133 | 0.044 | 93.2 | 0.003 | 0.001 |
| 400 | 0.148 | 0.151 | 0.156 | 0.152 | 0.025 | 90.5 | 0.004 | 0.001 |
| 200 | 0.163 | 0.166 | 0.165 | 0.165 | 0.011 | 88.6 | 0.002 | 0.000 |
| 100 | 0.178 | 0.177 | 0.174 | 0.176 | 0.009 | 87.6 | 0.002 | 0.001 |

***Table 6. Effect of extract Anacyclus clavatus flowers on HRBC hemolytic and membrane stabilization***

| Sample (4) Conc. ug/ml | OD R1 | OD R2 | OD R3 | Hypotonic Ab. Mean | Sample with Isotonic solution Ab. | Hemolysis Inhibition % | SD | SE |
|---|---|---|---|---|---|---|---|---|
| Control | 1.115 | 1.112 | 1.118 | 1.115 | 0 | 0 | 0.003 | 0.001 |
| 1000 | 0.029 | 0.027 | 0.03 | 0.029 | 0.028 | 99.9 | 0.002 | 0.000 |
| 800 | 0.041 | 0.044 | 0.045 | 0.043 | 0.021 | 98.3 | 0.002 | 0.001 |
| 600 | 0.058 | 0.056 | 0.053 | 0.056 | 0.017 | 97.1 | 0.003 | 0.001 |
| 400 | 0.074 | 0.069 | 0.069 | 0.071 | 0.011 | 95.6 | 0.003 | 0.001 |
| 200 | 0.095 | 0.097 | 0.1 | 0.097 | 0.005 | 93.2 | 0.003 | 0.001 |
| 100 | 0.108 | 0.11 | 0.104 | 0.107 | 0.002 | 92.2 | 0.003 | 0.001 |

***Anti-Cancer Studies***

The results of anti-cancer activity were shown in the Table (7). The anti-cancer activities of the plant extracts and their nanoparticles were applied on Cancer cell lines (HePG2). The plant extract was shown to have potential antitumor action against cell lines. The cytotoxicity of the plant extract was tested using human Liver (HepG 2) cell lines at five different doses (31.25, 62.5, 125, 250, 500 and 1000 µg/mL). The MTT test showed that expanding the grouping of the plant separate diminished cell suitability and expanded cytotoxicity. The IC50 values of plant extract were reported to be 85.75 and 388 µg/ml for leafs and stem extracts; respectively (Table 7 & Figures 7-8).

The results of this study discovered that when concentrations increase, cell growth inhibition (cytotoxicity) and cell viability decrease. The findings of this research correspond with those of some studies [25], showed that the extract fractions of some plants cause HepG 2 cells to undergo apoptosis at increasing concentrations, indicating that these fractions might be utilized to create anticancer compounds. Examining the morphology, the extracts removal affects the morphology of HepG2 cells following treatment with different concentrations. When the concentration of plant extract (up to 250 µg/mL) was raised, the morphological parameters of the tested cell lines altered substantially, and this change was proportional to the applied concentration. They started to shrink and lose their capacity to stick to the culture plate's surface as a result. Furthermore, in compared to the control morphology, cells seemed rounded and totally floated at the maximum applied concentration.

Inducing cell toxicity and apoptosis (morphology change) in HepG 2 cells has anticancer action. Cell lines treated with varying concentrations of extracts concentrate were compared to untreated cells over the course


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of a 24-hour period. When contrasted with untreated malignant growth cells, the state of plant separates treated disease cells changed extensively. The extricate treated cells had lost their film honesty, despite the fact that it was as yet flawless at lower focuses. At more prominent dosages, in any case, the extracts treated cells uncovered a critical contrast from the 47-benchmark group. Nuclear chromatin condensation and aggregation, as well as apoptosis, started to occur when cells shrank and lost their capacity to adhere to the culturing plate. These morphological alterations in cell lines seen in the study were consistent with earlier research suggesting that this kind of change in cells might be a potential cause of cell death [26]. In contrast to the control morphology, which was proportionate to the applied concentration, the examined cell lines' morphological features were significantly altered, and they were now completely afloat.

***Table 7. The toxicity and viability values of anticancer investigations:***

| ID | ug/ml | O.D | | | Mean O.D | ±SE | Viability % | Toxicity % | IC50 ± SD |
|---|---|---|---|---|---|---|---|---|---|
| HepG2 | -------- | 0.733 | 0.752 | 0.741 | 0.742 | 0.005508 | 100 | 0 | ug |
| 1 | 1000 | 0.022 | 0.019 | 0.02 | 0.020333 | 0.000882 | 2.74034142 | 97.25965858 | 85.75 ± 0.86 |
| | 500 | 0.02 | 0.02 | 0.022 | 0.020667 | 0.000667 | 2.785265049 | 97.21473495 | |
| | 250 | 0.042 | 0.038 | 0.039 | 0.039667 | 0.001202 | 5.34591195 | 94.65408805 | |
| | 125 | 0.116 | 0.153 | 0.132 | 0.133667 | 0.010713 | 18.01437556 | 81.98562444 | |
| | 62.5 | 0.487 | 0.492 | 0.476 | 0.485 | 0.004726 | 65.3638814 | 34.6361186 | |
| | 31.25 | 0.741 | 0.748 | 0.736 | 0.741667 | 0.00348 | 99.95507637 | 0.04492363 | |
| 2 | 1000 | 0.021 | 0.027 | 0.026 | 0.024667 | 0.001856 | 3.324348607 | 96.67565139 | 388 ± 1.56 |
| | 500 | 0.176 | 0.193 | 0.18 | 0.183 | 0.005132 | 24.66307278 | 75.33692722 | |
| | 250 | 0.653 | 0.661 | 0.643 | 0.652333 | 0.005207 | 87.91554358 | 12.08445642 | |
| | 125 | 0.736 | 0.74 | 0.739 | 0.738333 | 0.001202 | 99.50584007 | 0.494159928 | |
| | 62.5 | 0.733 | 0.752 | 0.738 | 0.741 | 0.005686 | 99.86522911 | 0.134770889 | |
| | 31.25 | 0.738 | 0.744 | 0.74 | 0.740667 | 0.001764 | 99.82030548 | 0.179694519 | |

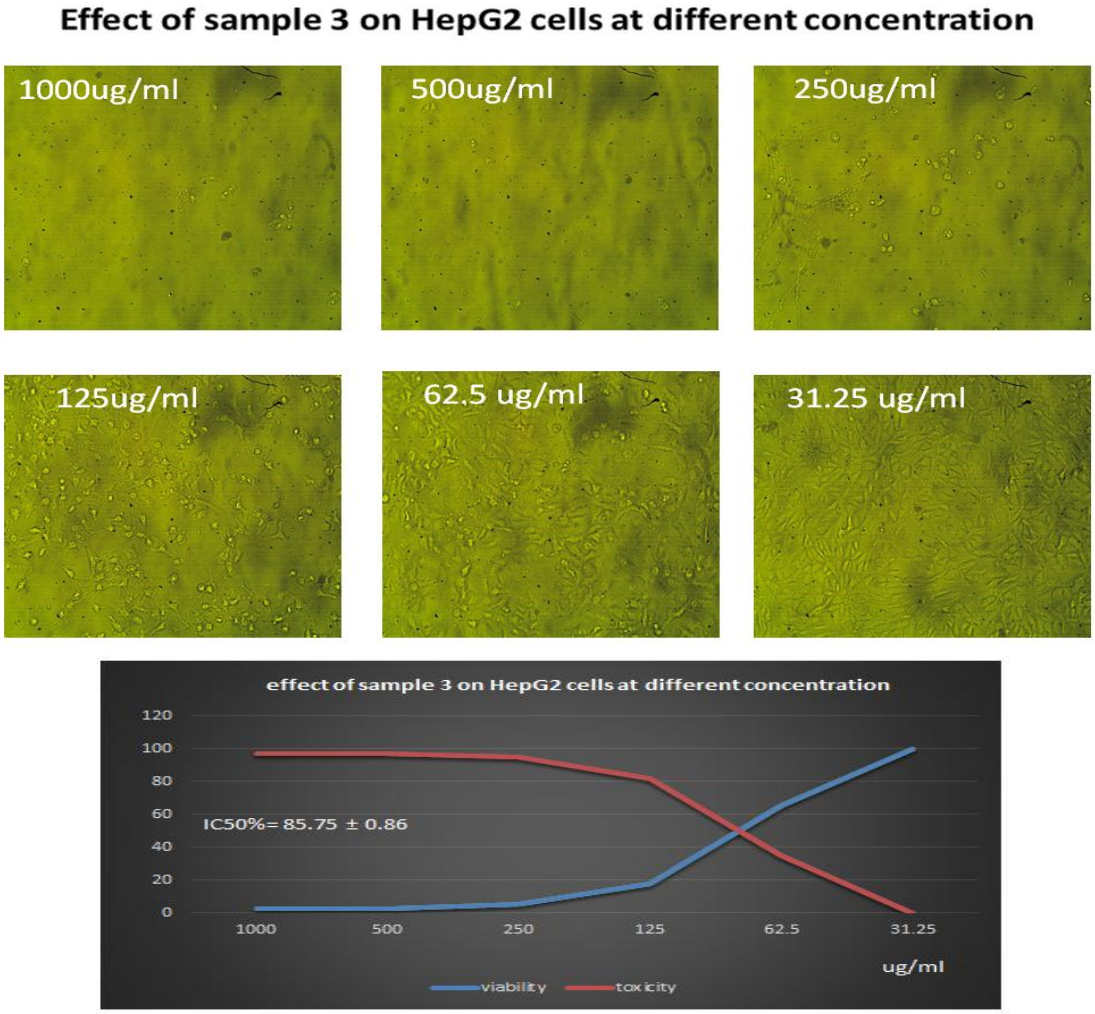


***Figure 7. Effect of Anacyclus clavatus stems (Sample 3) extract on HepG2 Cells at different concentrations***


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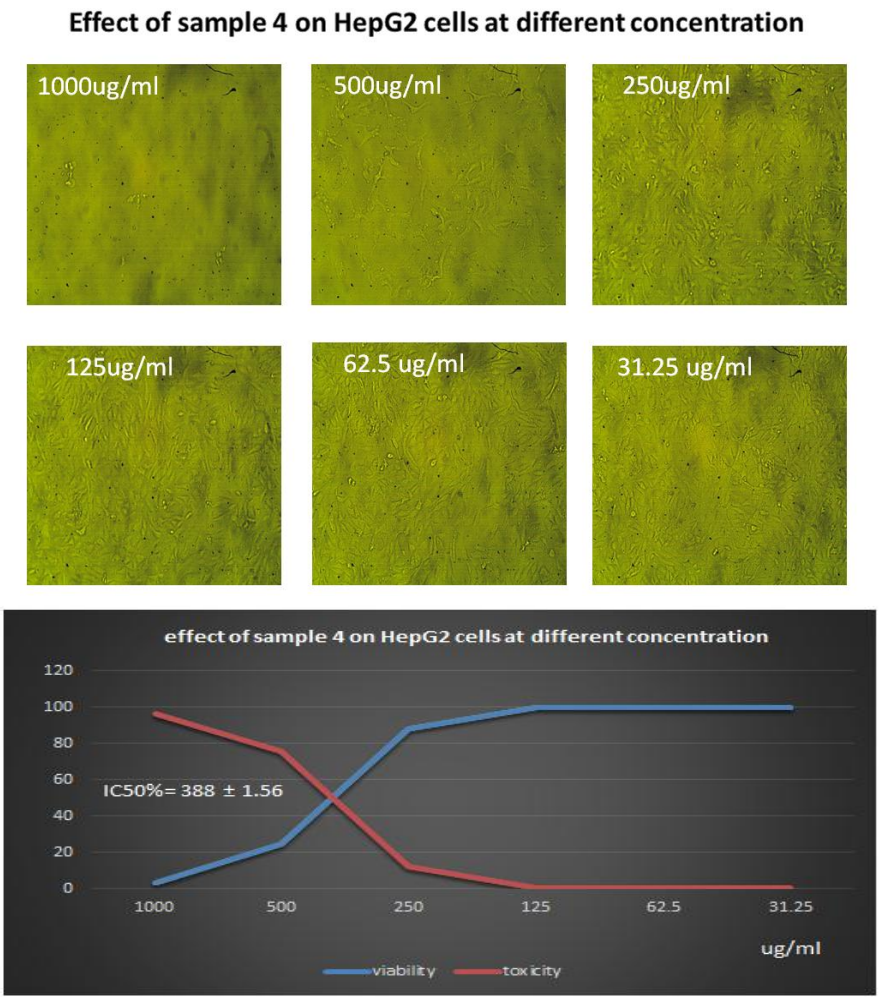


***Figure 8. Effect of Anacyclus clavatus flowers (Sample 4) extract on HepG2 Cells at different concentrations***

## Discussion

This study investigated of the antioxidant properties and total phenolic contents of the selected Anacyclus *Clavatus* plant extracts. Phenolic levels are high in both extracts. Higher extract concentrations than the control resulted in high inhibition of 87.6–99.5%. The extracts of different parts of *Anacyclus Clavatus* were applied, they showed predominantly bactericidal effect, and differences exist between strains, as well depending on the extract and part of the plant used. Our findings showed that the ethanolic extract of *Anacyclus Clavatus* had a greater antibacterial inhibitory effect than the aqueous (water) extract against *Staphylococcus aureus*. The findings of this study are consistent with findings about the antibacterial properties. Some studies showed that the extracts had decreased anti-Klebsiella *spp* action [27]. Similar results have been documented for extracts of plants Capable of inhibiting Staphylococcus aureus but just minimally impacting *E. coli.* [28]. Anti-inflammatory drugs mostly work by converting arachidonic acid to prostaglandins, which inhibits the cyclooxygenase enzyme that produces inflammatory mediators. The peroxidase enzyme forms long membrane channels by converting prostaglandin G2 (PGG2) into PGH2. Arachidonic acid was released from the membrane and transformed into prostaglandin as a result of the opening of the membrane channel brought on by the release of chemical mediators. The extracellular activity of these enzymes is thought to be associated with both acute and chronic inflammation. [29] Assessed the anti-inflammatory effects of some plants in vitro through observation of the inhibition of protein denaturation and the HRBC membrane stabilizing assay. Therefore, compared to some research, the current study's percentage of inhibition is higher.

This study evaluated if *Anacyclus Clavatus* extracts can kill cancer cells in a lab environment. To evaluate cell death, researchers employed an MTT assay. In this assay, a positively charged MTT dye transforms into a purple Formosan product when it enters living cells that are actively metabolizing. Since healthy cells have higher levels of metabolic activity, the amount of formazan reflects cell viability ]30]. The results of this study correspond with some studies,[25], which demonstrated that HepG 2 cells suffer apoptosis in response to extract fractions from specific plants at increasing concentrations, suggesting that these fractions could be used to make anticancer drugs


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The results obtained in this study showed that HepG2 cells significantly decreased their proliferation when treated with *Anacyclus Clavatus* plant extracts and that untreated HepG2 cell lines contained fewer dead cells than cells of the treated group. A study stated that [31], due to found a 15 kDa protein in *Anacyclus Clavatus* which triggers mouse cancer cells to activate caspase 3. Significantly, this protein also raised the expression of the Bcl-2 gene, indicating that the plant extract might have complex apoptotic effects. The inflammatory response in HCC is inhibited by extracts due to how they block NF-kappaB and MAPK activity in RAW 264.7, which draws lipopolysaccharide [32].
In this study the antioxidant capacity was estimated, because it is a marker of oxidative stress for cancer [33]. Previous studies [34] reported that the phenolic chemicals are responsible for the anti-cancer activity. As a result, the findings provide an adequate basis for further research into the possible applications of *Anacyclus Clavatuss* in both the food and medical industries. Apoptosis-inducing natural bioactive chemicals found in medicinal plants could be a viable new source of anticancer medications.

**Conclusion**

The purpose of this study is to estimate the anti-cancer potential of two parts of the *Anacyclus Clavatus* extracts were prepared using ethanol and water solvents and tested on HepG2 cancer cells to evaluate their antioxidant and anti-cancer effects. The anti-cancer potential was assessed using standard methods, and cell viability was measured using MTT assays to quantify cell death and viability. In comparison to the control group, HepG2 cells treated with plant extracts showed reduced viability, proliferation, and a rise in apoptosis, or programmed cell death. Cancer cells treated with plant extracts have stronger antioxidant activity than untreated cancer cells. Using extract has stronger anti-cancer and antioxidant ability against cancer cells. Extracts of *Anacyclus Clavatus* have been found to induce anticancer activity in cancer cell lines by increasing apoptosis and antioxidant status and reducing proliferation. The results of this study showed that the *Anacyclus Clavatus* plants contains a number of Phytoconstituents, which reveals its uses for different therapeutic purposes. The leafs and stems can be used for the treatment of various disorders in human being such as inflammatory, antimicrobial activity, antioxidant and anti-cancer. Using plant extracts for medical applications is highly recommend as alternative drugs, also using other applications as inflammatory is very important in future studies. The detection on the types of phenolic acids by GC-Mass analysis is very important to evaluate the types and contents on phenolic acids. Still more work is required with the *Anacyclus Clavatus* to investigate the mechanism of actions with other therapeutic activity.

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***Conflict of interest***. Nil

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**المستخلص**

تم استخدام الطب البديل بالأعشاب في ليبيا منذ العديد من السنوات السابقة بليبيا وذلك لكونها تعتبر من الاكثر المناطق احتواء عللا النباتات الطبية وخصـوصـا بمنطقة الجبل الاخضـر بليبيا ، وتهدف هذه الدراسـة الي تحديد محتويات كلا من مضادات الأكسدة والمحتوى الكلي من المركبات الفينولية وكذلك الفحص الفيتو كيميائي ومدى فعالية مضادات البكتريا والفطريات والالتهابات والخلايا السرطانية لمستخلصات سيقان وزهور نبات القريصة النامية في الجبل الاخضر. وقد بينت نتائج هه الدراسـة وجود العديد من المركبات الطبيعية الهامة مثل الكربوهيدرات او الجليكوسـيدات والسـيترولات او التربينات والتانينات والفلافونيدات في مسـتخلصـات النبات قيد الدراسـة. وكانت تراكيز المركبات الفينولية الكلية 9.1 و 11.40 جزء من المليون ، بينما كانت محتويات مضادات الاكسدة 3.26 , 3.93 جزء من المليون ، و ذلك لمستخلصات السـيقان والاوراق على التوالي. كما بينت نتائج هذه الدراسـة ان المسـتخلصـات اعطت تأثيرا مثبطا على انواع البكتريا المستخدمة في هذه الدراسة وهي (Staph and Klebsiell)والفطريات (Aspergilluis and Penicillium) ، وكذلك اعطت تأثيرا مثبطا على الالتهابات ، وتم اسـتخدام المسـتخلصـات كمضـادات للخلايا السـرطانية على خلايا سـرطان الكبد وبينت النتائج ان هناك تأثيرات عالية عليها.


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