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Some Chemical Studies on Cytotoxicity of Antioxidants from Propolis Extracts against Human Pancreatic PANC-1 Cancer Cell

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Abstract

Propolis is a resin-rich in a bioactive compound that bees collect from plants. Propolis extracts, especially Nano particle extraction, show promising importance in health and disease treatment. In this study, total phenolic and total flavonoids for both Nano-propolis (NP) and water extract of Egyptian Propolis (WEP), Antioxidants activity (AOA%), cytotoxicity to pancreatic cancer cell line PANC-1. NP was characterized using TEM, Zeta sizer, Zeta potential, and UV-vis spectrophotometer. Total flavonoids, Total phenolic, and antioxidant activity of NP were significantly greater than WEP, whereas NP and WEP showed Total flavonoids 36.09, 5.55 mg/g, Total phenolic 7.12, 5.23 mg/g, and AOA% 30.97%, 29.05% respectively. Likewise, the cytotoxicity of NP and WEP against PANC-1 showed IC_{50} 26.4±2.1 and 117±7.2 µg/ml, respectively. In addition, the cytotoxicity of NP and WEP against normal human lung fibroblast cells MRC-5 showed CC_{50}; 61.7±5.3 and 232±11.8 µg/ml. These results could be related to an infinite variety of secondary metabolites compounds from plants in Propolis. Overall, these results provide important insight into the value of Nano-propolis as an interesting natural source for anticancer treatment.

Keywords: Propolis; Nano-propolis; Water extract of Propolis; Total flavonoids;Total phenolic; Cytotoxicity; Pancreatic cancer cell line.

1. Introduction

Compounds contained in over 70% of natural anticancer products are highly useful in cancer treatment. Molecular tweaking of natural chemicals can also boost their medicinal value. More effective targeted therapeutics can be achieved by conjugating harmful natural compounds to polymeric carriers or monoclonal antibodies. Research into natural products use as chemotherapeutic agents requires the scientists' participation from a wider range of disciplines [1]. Since only 15% of higher plants have been studied.

Propolis is a complex compound made mostly of waxes, resin, and volatiles. Depending on the plants that were used to extract the resinous substance, its color might range from yellow to brown to even black. Bees make Propolis from tree and shrub resin by mixing it with enzyme-rich saliva and beeswax [2]. Propolis has a strong, pleasant fragrance [3]. Many other chemical groups may be found in propolis resin, but the most common ones include flavonoids (including flavones, dihydroflavonols, chalcones, and flavanones), aromatic aldehydes, terpenes, alcohols, stilbenes, fatty acids, and b-steroids [4, 5]. The ancient Egyptians employed Propolis as an embalming agent because it served as a plastic that warded off microorganisms [3, 4]. Immunomodulatory, antioxidant, anti-inflammatory, antibacterial, antiviral, antiparasitic, and antifungal are only some of the biological functions Propolis may do [2]. It has only been lately that propolis' anticancer activity was examined [5].

Propolis consists of numerous chemical compounds such as polyphenols, terpenoids, amino acids, sugars, steroids, minerals, and vitamins. Polyphenols, such as flavonoids, phenolic acids and their esters, phenolic alcohols, aldehydes, and ketones are important groups of propolis constituents, due to their wide biological activity [6],[7]. Flavonoids, such as galangin, apigenin, pinocembrin, pinostrobin, kaempferol, chrysin, and quercetin, and also phenolic acids,

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including caffeic, ferulic, cinnamic, coumaric, and hydroxybenzoic were determined in propolis samples from various geographic origin [8].

Most recent research has focused on the health aspects of flavonoids for humans. Many flavonoids are shown to have antioxidative activity, free radical scavenging capacity, coronary heart disease prevention, hepatoprotective, anti-inflammatory, and anticancer activities, while some flavonoids exhibit potential antiviral activities. In plant systems, flavonoids help in combating oxidative stress and act as growth regulators. For pharmaceutical purposes, cost-effective bulk production of different types of flavonoids has been made possible with the help of microbial biotechnology [9].

Nano-propolis is a kind of Propolis where the particles are joined together at the nanoscale, making the final product more effective without compromising any of the original substance's unique qualities. Consuming free-form propolis, which has poor bioavailability, solubility, absorption, and untargeted release, will result in a reduction of the advantages previously mentioned. Several distinct approaches to nano-encapsulation are used during the collection process of nano-propolis, where the human body more easily absorbs them than traditional propolis because of their smaller size. When compared to the antimicrobial qualities of nano-propolis, the antifungal and antibacterial capabilities of propolis come in second place [1].

The most important evaluation for nanoparticles is known by its technical term, zeta potential, which mentions to the electrokinetic potential in colloidal systems. because of its profound impact on the many characteristics of Nano-drug delivery systems. Assigning an electrokinetic value to a surface charge consistent with reality yields this result. Colloidal Nanocarriers are expanding rapidly because of their promising potential to address persistent problems, such as low medication solubility and bioavailability. In addition, their potential for therapeutic targeting appears to be boundless [10].

Cancer diseases are defined by the unrestrained proliferation and spread of abnormal cells; it contains hundreds of subtypes, each of which has its own distinct risk factors and survival rates [11]. Cancer is a disease that may affect any part of the body. When measured by the economic cost of mortality and disability, cancer is second only to cardiovascular disease as a primary cause of death globally [12]. Cancer patients now have a far better chance of survival because of advances in therapy and earlier detection [13]. Flavonoids have been consumed by humans to have extensive biological properties that promote human health and help reduce the risk of diseases. Oxidative modification of LDL cholesterol is thought to play a key role during atherosclerosis [14],[15]. Mechanisms of antioxidant action can include (1) suppression of ROS formation either by inhibition of enzymes or by chelating trace elements involved in a free radical generation; (2) scavenging ROS; and (3) upregulation or protection of antioxidant defences [16].

There has to be more research done on Propolis and its constituents in terms of its antitumor and immunomodulatory effect in vivo. Several isolated chemicals from Propolis have been studied for their potential role in the substance's anticancer activity. Despite this, additional chemicals and the additive effects of these compounds have to be examined in tumour research both in vivo and in vitro. This is because the composition of propolis is rather intricate. It has been shown that the immunomodulatory activity of propolis, both in vivo and, in vitro may have a favourable impact on the chemoprevention of cancer [1]. In this impact, the activation of humoral immunity, the increase of cell-mediated immunity, and the stimulation of non-specific immunity are included.

Numerous epidemiological and preclinical studies suggested that; the immune system plays a major role in the cancer prevention. As a result, there has been an increase in the popularity of propolis as a means to reduce the incidence of cancer. It has been shown that propolis has an anticancer effect; however, the mechanism by which this happens is not completely known. Multiple studies have shown that the primary mechanisms of anticancer that propolis possesses are the inhibition of matrix metalloproteinases, the prevention of angiogenesis and metastasis, the arresting of the cell cycle, and the activation of apoptosis. Propolis is an extract from the resin of the propolis tree. Both rats and humans showed no signs of systemic toxicity or deleterious effects when propolis was given to them in vivo [17]. Additionally, the propolis extract can be suppress the tumour cells growth of the SF-295 (human glioblastoma), HCT-116 (colon), and OVCAR-8 strains at a rate that was higher than 75% (breast). The key chemical components responsible for these biological effects have been identified as flavanones, xanthones, flavanols, Chalcones Aurones, Catechins, and leucoanthocyanidins [18].

The current study aimed to determine total phenolic, antioxidant activity, flavonoids compounds, and identification of phenolic compounds fractionation by (HPLC), also evaluation the cytotoxic assay on pancreatic carcinoma cell lines (PANC-1) and Normal human Lung fibroblast cells (MRC-5) of Nano-propolis (NP) and water extract of Egyptian Propolis (WEP).

2. Materials and methods

This study set out in 2020 to assess the importance of propolis antioxidants NP and WEP in treating pancreatic cancer cell lines PANC-1.

Characterization of Nano-propolis (NP)

Using a Zetasizer (Nano-ZS; Malvern Instruments, UK), that based on dynamic light scattering (DLS) and laser Doppler electrophoresis, polydispersity index (PDI), the particle size, Zeta-potential of vacuum, and PE-loaded NP were determined at 25 degrees Celsius. An Indonesian company called good fit was the one that provided the NP. For determining the wavelength absorption, a Cary 5000 UV-Vis-NIR spectrophotometer was employed. The morphological characteristics of the newly generated NPs and NIMs with the use of a transmission electron microscope (TEM) that has high-performance digital imaging capabilities (GEM-1010; JEOL, USA) were examined. After coloring the particle suspension, a single drop was spread on a carbon-coated copper grid so that the color could be seen. The sample was dried at room temperature throughout the process. The specimens were then deposited at Regional Centre for Mycology and Biotechnology TEM for imaging at 70 kV accelerating voltage, Al- Azhar Uni. [19],[25].

Water Extraction of Egyptian Propolis (WEP)

Brown bee propolis powder from Egypt was on the market (Giza, Egypt). For 15 minutes at 70 °C, 10 grams of powdered Egyptian Propolis was heated with 20 grams of polyethylene glycol (PEG) 400 (Alpha chemical-India). The cloudiness was removed from the propolis extracts by passing them through Whatman No. 1 filter paper [19].

Determination of total phenolic compounds

The Folin - Ciocalteau reagent was used to do an analysis on the total phenolic compounds (Sigma-Aldrich). In a test tube, the following ingredients were combined: 500 ml of a known concentration of an extract solution, 2.5 ml of a Folin-Ciocalteu reagent that had been diluted by a factor of 10, and 2 ml of 7.5% sodium carbonate. After finishing the wrapping of tubes, they were let to sit at room temperature for half an hour. The concentration of gallic acid was compared to a standard curve, which was plotted against the data. Spectrometric measurement of the absorbance was done at a wavelength of 760 nm. The findings were presented as the quantity of gallic acid expressed in milligrams for each individual unit of sample weight [20].

Determination of total flavonoids

The total of content flavonoids was analysed regarding to Zhishen [21], with slight modification. First, 500 µL of sample extract was combined with 2 mL Dist. H_2O, and then 150 µL of 5% sodium nitrate solution was added. After 6 minutes, 150 µL of 10% $AlCl_3$ was added. The mixture was let to stand for 6 minutes. Following that, 2 ml of NaOH at a concentration of 4 % was added to the mixture. During the same time, Dist. H_2O was added to reach total volume 5 ml. After that, the ingredients were combined and let to rest for 15 min. At 510 nm, Spectrophotometric analysis was used to compare the obtained absorbance to a blank value. The findings were reported in mg of quercetin/g of the sample's dry weight. There was a transaction that took place to acquire the flavonoid reference standard (Merck-Schuchardt).

Determination of antioxidant activity percentage (AOA%)

The activity of the antioxidant was measured by the samples' ability to quench 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich) radicals using the colorimetric method as mentioned before Brand-Williams [22]. A sample extract of 100 ml was added to 4 ml of DPPH solution (6×10−5M in MeOH). After that, the components were combined and let in the dark at room temperature for 30 min. The finished solutions were analysed by measuring their absorbance at 517 nm using a Spectrophotometer. The proportion of DPPH radical inhibition by each sample was determined using [23].

Inhibition% = (Ac (0) – AA (t)) / Ac (0) × 100

Where:

Ac (0) is the absorbance of the control at time = 0 min.

AA (t) is the absorbance of the antioxidant at time =1hr.

Fractionation of Phenolic compounds

High-performance liquid chromatography (HPLC) was used to isolate and identify phenolic compounds, as detailed in [24]. This study made use of an Agilent 1260 HPLC system. The separation was done using a 5 m long, 4.6 mm in diameter, 250 mm internal diameter, Eclipse Plus C18 column. At a 1 ml/min flow rates, the mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–5 min (80% A); 5-8 min (60% A); 8-12 min (60% A); 12-15 min (85% A) and 15-16 min (82% A). The multi-wavelength detector was monitored at 280 nm. Every one of the sample solutions was injected with a volume of 10µl. The temperature within the column was kept at 35±1°C. To calibrate the HPLC, a known concentration of phenolic acid (Sigma-Aldrich) was diluted in a mobile phase and injected. Data analysis using Hewlett Packard Software determined the concentration of phenolic chemicals based on retention time and peak area.

Evaluation of Cytotoxic Effects of NP and WEP on PANC-1 and MRC-5

Mammalian cell lines: Pancreatic carcinoma cell lines (Panc-1 cells) were got from the American Type Culture Collection (ATCC, Rockville, MD).

Mammalian cell lines: Normal human Lung fibroblast cells (MRC-5 cells) were got from Tissue Culture Unit, VACSERA, Giza, Egypt.

Chemicals Used:

For this experiment, DMSO, MTT, and trypan blue (Sigma-Aldrich) were used. Also, Dulbecco's Modified Eagle's Medium (DMEM), fatal bovine serum, Roswell Park Memorial Institute (RPMI)-1640 (RVI), HEPES buffer solution (pH 7.4), L-glutamine (L-Gln), gentamycin (Lonza, Belgium) were used. For the crystal violet stain (Sigma-Aldrich), 0.5 percent (w/v) crystal violet with 50 percent (v/v) methanol were combined. The mixture was reached to the target volume using ddH2O, and then filtered using filter paper Whatman No.1.

Cell line Propagation:

Pancreatic adenocarcinoma (PANC-1) cells were cultured in RPMI-1640 media with 10% inactivated fetal calf serum and 50 g/ml gentamycin. 1% L-glutamine, HEPES buffer, Dulbecco's modified Eagle's medium (DMEM) with 10% heat-inactivated fetal bovine serum, and 50g/ml gentamycin were used to cultivate MRC-5 cells. Each kind of cell was subcultured twice weekly and kept at 37 ºC in a humidified environment containing 5 % carbon dioxide.

Cytotoxicity evaluation

Cytotoxicity was evaluated using viability assay. Aspirated material from the MRC-5. Corning® 96-well tissue culture plates were seeded with tumor cell lines in RPMI-1640 media at concentrations of 5x104 cell/well for PANC-1 and 1×104 cell/well for MRC-5 for 24 hours before being placed in a humidified incubator with 5% CO_2. 1% crystal violet solution was added in each well for at least 30 minutes. The plates were then cleaned under running water to eliminate any remaining traces of stain. The absorbance was measured at 490 nm after being gently shaken and then treated to a final addition of glacial acetic acid (30 %) in each well.

However, following PANC-1 incubation, the media was withdrawn from the 96-well plates and replaced with 100 µl of new RPMI 1640 medium without phenol red. Finally, 10 µl of the 12 mM MTT stock solution (5 mg of MTT in 1 mL of PBS) was added to each well, including the untreated controls. The 96-well plates were kept in an incubator for 4 hours at 37 °C with 5% CO_2. After removing an aliquot (85 µl) of the medium from each well, 50 µl of DMSO was added and properly mixed with the pipette before being incubated at 37°C for 10 minutes. Using a microplate reader, the optical density was determined at 590 nm (Sunrise, TECAN, Inc, USA). To determine the proportion of viable cells, the experiment was repeated three times and used the following equation.

Cell viability (%) = $\frac{\text{OD treatment}}{\text{OD control}} \times 100$.

Graphical representations of the dose-response curve for each concentration were used to determine the cytotoxic concentration (CC_{50}) and the inhibitory concentration (IC_{50}), both of which are the concentrations needed to elicit harmful effects in 50% of intact cells. Making use of GraphPad Prism (San Diego, CA. USA) [26]

Statically analysis

The obtained data were subjected to statistical analysis of variance. The means were compared using the "Least Significant Difference (LSD)" test at the 5% level as described by Steel and Torrie (1980) [27].

3. Results and discussion

Physicochemical properties, such as shape, particle size, and surface charge, play a principal role in the cellular uptake of nanoparticles. Cell nanoparticle uptake can be considered a two-step process: The first step is binding on the cell The membrane. The second step is the internalization step [28].

Image 1: The Nano-propolis (NP) particles (100 nm, TEM mag = 100000x).

The imaging of nano-propolis with a transmission electron microscope is one piece of experimental proof for the characteristics of nanoparticles (TEM) because the pictures and sizes are acquired by transmitting the particles' entire surface, and images (1). The mean size particle distribution's ±(SD) was 15.77±9.33nm, Image (1) displays the Nanoparticles' size and shape as seen through TEM (at a magnification of 100,000x); Figure (1) displays the same information for a Zeta sizer nano, which revealed a particle size of 11.67 nm and a wavelength of Abs 290 nm (3). [29]

Nano-carriers need to be characterized once they have been prepared to ensure they are fit for in vitro and in vivo use. The polydispersity index is a metric used to indicate the range of size of the lipidic nano-carrier systems in terms of particle size distribution characterization (PDI). The degree to which a size distribution of particles is not uniform is referred to as its "polydispersity" (or "dispersity," as suggested by IUPAC). PDI, also known as the heterogeneity index, measures how dissimilar the correlation data is (the cumulants analysis). [30]

A sample's PDI represents the distribution of the sample's size as a whole. If the particles in a given sample are all the same size, then the PDI value will be zero (for a highly polydisperse model with multiple particle size populations). In reality, acceptable values for polymer-based nanoparticle materials are often below 0.2. A PDI of 0.3 or below is deemed acceptable and shows a homogeneous population of phospholipid vesicles for applications of drug delivery employing lipid-based carriers as liposomes and nanoliposome formulations. With a PDI of 0.246, we find a consistent number of phospholipid vesicles in our sample [30].

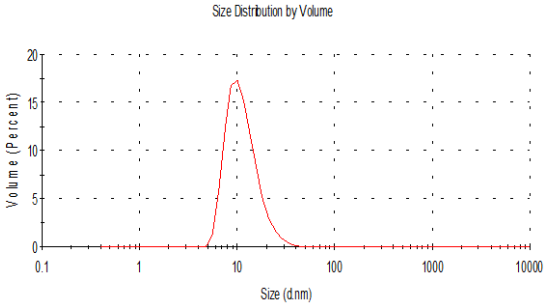

Fig. 1:Polyphenols content of NP using HPLC
Fig.1 size distribution by volume

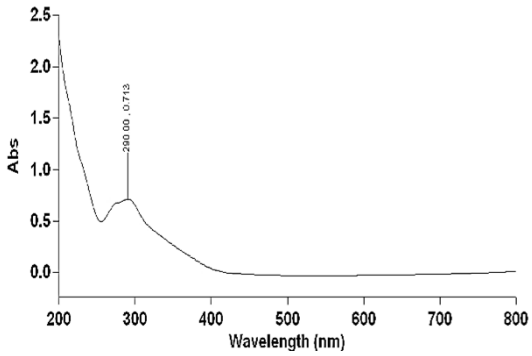

Fig. 2: Wavelength (Abs) of NP

Carcinoma cells contain extensive negatively charged domains in their cell membranes [9], which should cause them to be repelled by negatively charged nanoparticles. The Zeta Potential (ZP) of NP is surprisingly low at -10.4 mV, as seen in Fig. (3) has a profound impact on pancreatic cancer, which may be described as follows: there are few cationic sites for negatively charged particles adsorption, and the negatively charged particles bind to the cationic sites in the form of clusters [31].

According to another study [32], the high cellular absorption of negatively charged nanoparticles was linked to the non-specific process of nanoparticle adsorption on the cell membrane and the creation of nanoparticle clusters. Electrostatic adsorption of negatively charged particles at positively charged locations might result in localized neutralization and, in turn, the bending of the membrane encouraging endocytosis for cellular absorption [33]. Once within a cell, nanoparticles make their way to specialized compartments called primary endosomes and sorting endosomes. Most nanoparticles are taken to secondary endosomes, although a small percentage is recycled back to the cell's outside via exocytosis. The effectiveness of cytoplasmic delivery is determined by how quickly and easily nanoparticles can leave the endo-lysosome formed when secondary endosomes merge with lysosomes. In addition, numerous studies have postulated and demonstrated that endolysosomal acidic pH is responsible for nanoparticle surface charge reversal, leading to their escape from the endolysosomal system [34].

Antioxidant activity (AOA%)

Total flavonoids, Total phenolic, and NP antioxidant activity were significantly greater than WEP, whereas NP and WEP showed Total flavonoids 36.09, 5.55 mg/g, Total phenolic 7.12, 5.23 mg/g, and AOA% 30.97%, 29.05% respectively (Table 1). The statistics for NP and WEP samples revealed that NP significantly increased WEP in total phenolic, flavonoids, and AOA percentage. The current results are due to the components varying significantly

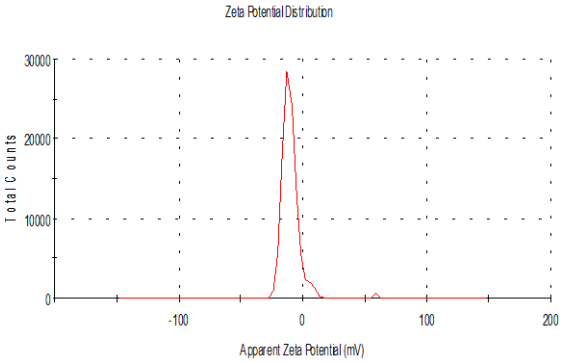

Fig. 3 zeta potential distribution

according to their geographical and botanical origins. Propolis significantly impacts the appreciation of its organoleptic character through its volatile fraction, which is one of the essential quality attributes [2].

Table 1: Total phenolic and flavonoids in NP and WEP samples

Samples	Total phenolic (mg/g)	Total flavonoids (mg/g)	AOA %
NP	7.12^{a}±0.148	36.09^{a}±0.180	30.97^{a}±0.174
WEP	5.23^{b}±0.112	5.55^{b}±0.127	29.05^{b}±0.118

Data shown in the column followed by different letters are significantly other at $p<0.05$ to the ANOVA test.

Figures (4) and (5) show the polyphenols content of NP and WEP by HPLC; the differences between NP

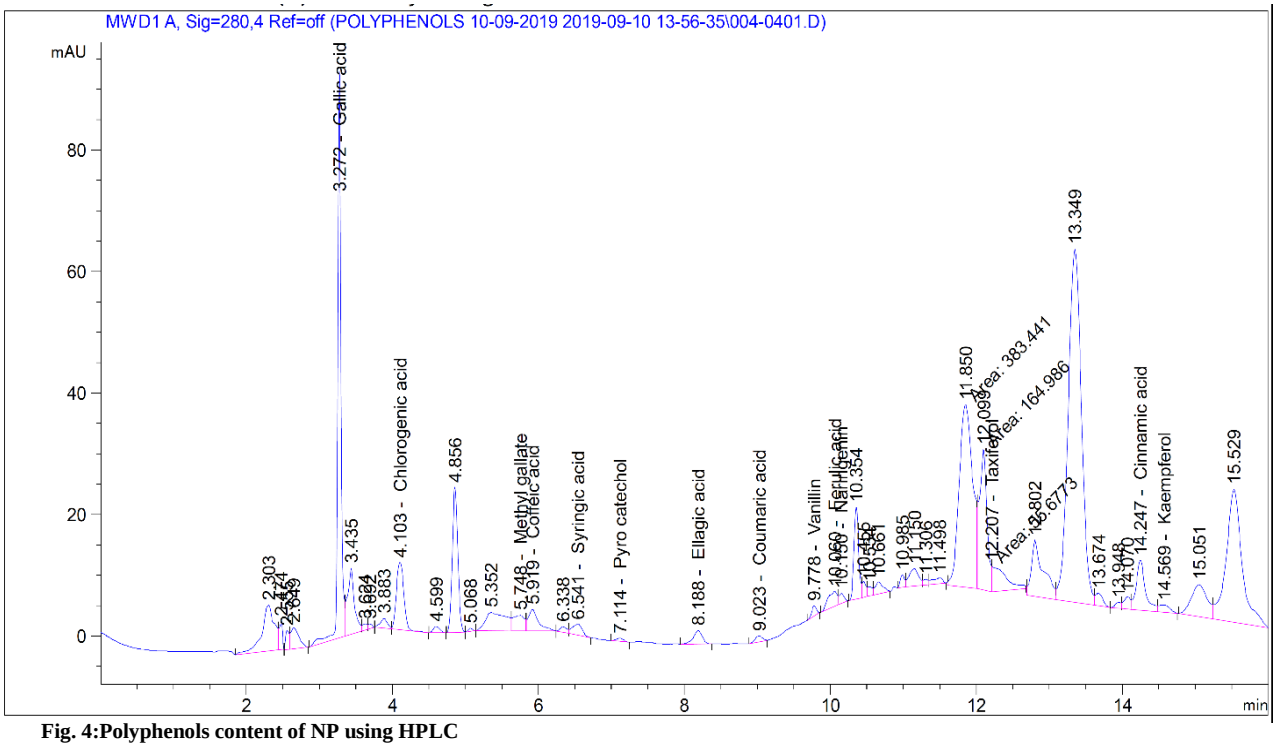

Fig. 4:Polyphenols content of NP using HPLC

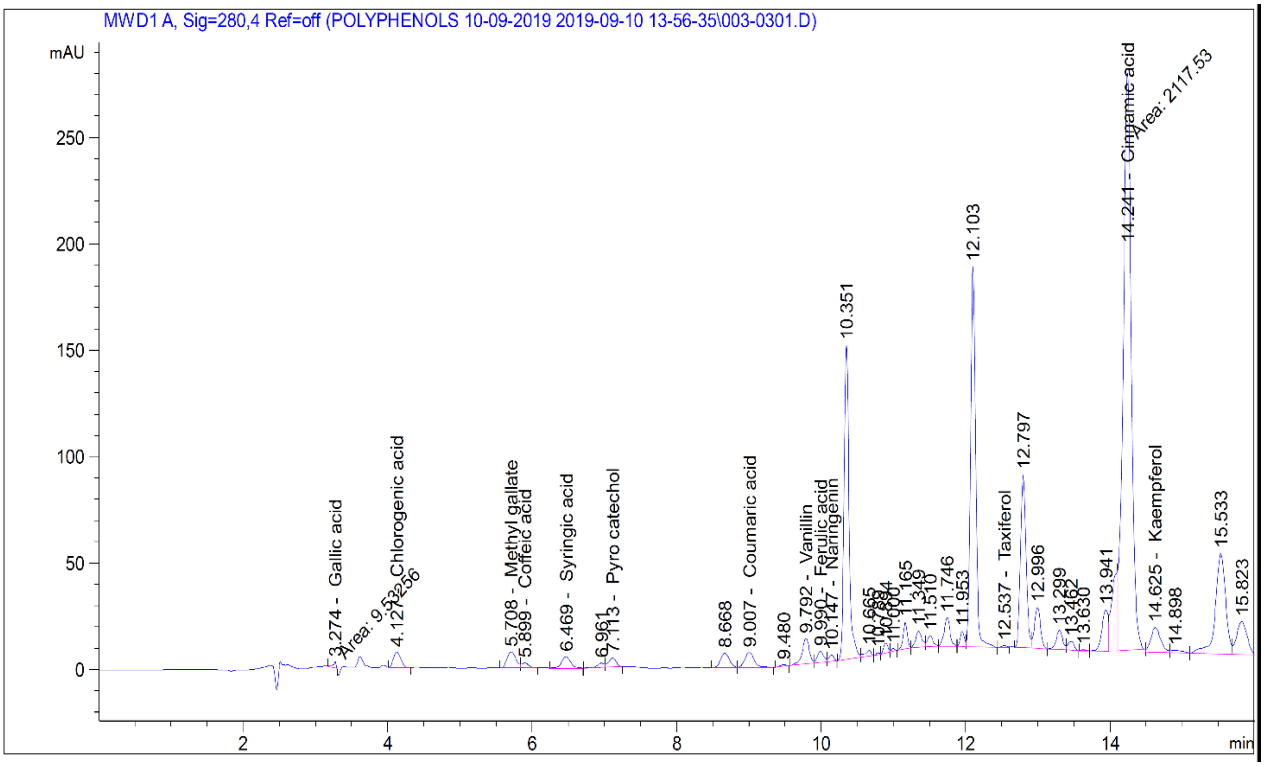

Fig. 5: Polyphenols content of WEP using HPLC.

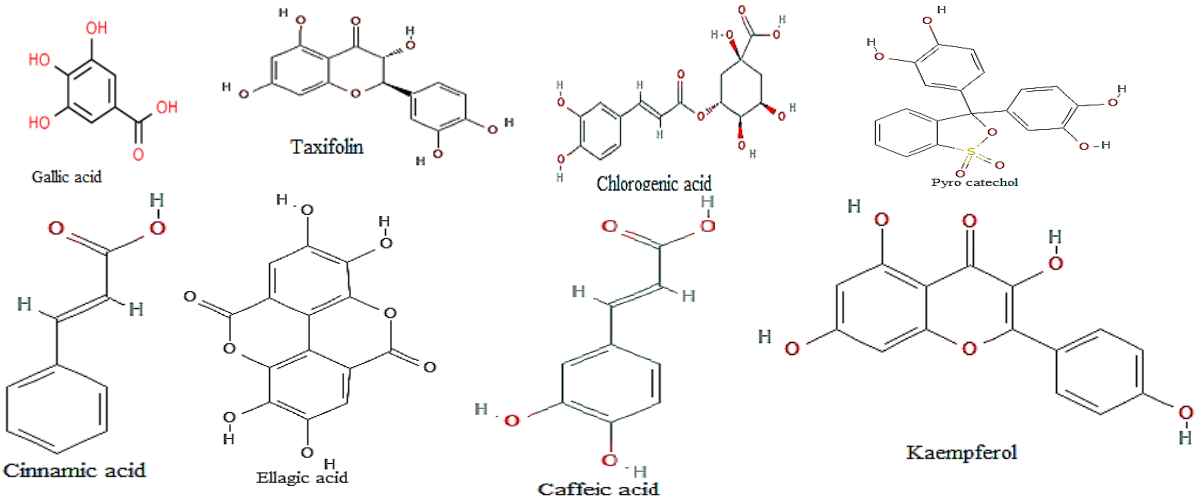

Fig. 6 the chemical structures of the analytes in propolis

and WEP were highlighted. NP has high concentrations (by calculating the compound area to standard) of Gallic acid, Chlorogenic acid, Taxifolin, and Ellagic acid with 219.58, 56.11, 49.30, and 10.06 µg/ml respectively, alternatively; WEP has high concentrations of Cinnamic acid, Kaempferol, Chlorogenic acid, and Pyrocatechol with 129.55, 32.16, 19.39, and 13.92 µg/ml respectively. the chemical structures of the analytes in propolis figures (6). As noted previously, Propolis's composition varied according to its geographical origins and source. The plants origin from which the resin is gathered, and the bee's kind affect the ratio of the various components found in propolis [2]. Poplar-type Propolis, which comes from climates that are more temperate and includes mostly phenolics such as aromatic acids, flavonoids aglycone, and their esters, is similar to Egyptian Propolis. However, the phenolics in tropical Propolis (such as Brazilian Propolis) vary. These include flavonoids, prenylated p-coumaric acid derivatives, lignans, benzophenones, and terpenes. As indicated above, Propolis's chemical composition and biological activity are affected by the geographical origin of the tree it was collected from.

Cytotoxicity activity

As mentioned before, the cytotoxic effect of NP and WEP on PANC-1 cells was investigated. The cells were treated with an array of concentrations (0, 1, 2, 3.9, 7.8, 15.6, 31.25, 62.5, 125, 250, and 500 µg/ml) of NP and WEP separately. After that, the cell viability was measured after 24 h. The in-vitro results showed that NP and WEP decreased the viability of PANC-1 cells in a dose-dependent manner. The IC50 values of NP and WEP on PANC-1 cells were determined to be 26.4-± 2.1and 117 ± 7.2 µg/ml, respectively (Figure 7). These results corresponded to Normal human lung fibroblast (MRC-5) as control. The inhibitory cytotoxic activity of NP against MRC-5 has been detected with CC_{50}= 61.7 ± 5.3 µg/ml. In addition, the Inhibitory cytotoxic activity of WEP against Normal human lung fibroblast cells was determined with CC_{50} =232 ± 11.8 µg/ml (Figure 8). Moreover, the current data indicates that the IC50 of NP and WEP on PANC-1 is about 42% and 50.4% of CC_{50} on normal cells MRC-5, respectively. One unanticipated finding was that NP is in the same line as IC_{50} of cisplatin and WEP are lower than of (Carboplatin) anticancer drugs compared with IC_{50} data of some Prestwick Chemical Library® (PCL) [11] From the current results of NP, which has higher Coffeic acid content more than 4 folds of WEP Surprisingly, the IC50 was observed to NP and WEP on PANC-1 was nearly the same folds. The current result is agreed with previous studies of the effect of caffeic acid cytotoxicity on leukemia [35] and the cytotoxic effect of Propolis [3].

The ability to induce apoptosis in cancer cells is a hallmark of successful anticancer treatment [36]. Apoptosis is defined as a form of programmed cell death that occurs at a genetic level. Propolis's ability to cause apoptosis appears to be chemical and extract concentration dependent. As a rule, apoptosis can occur via one of two distinct mechanisms. The first is triggered by tumor necrosis factor (TNF) receptors such as F as (TNF receptor superfamily member 6),

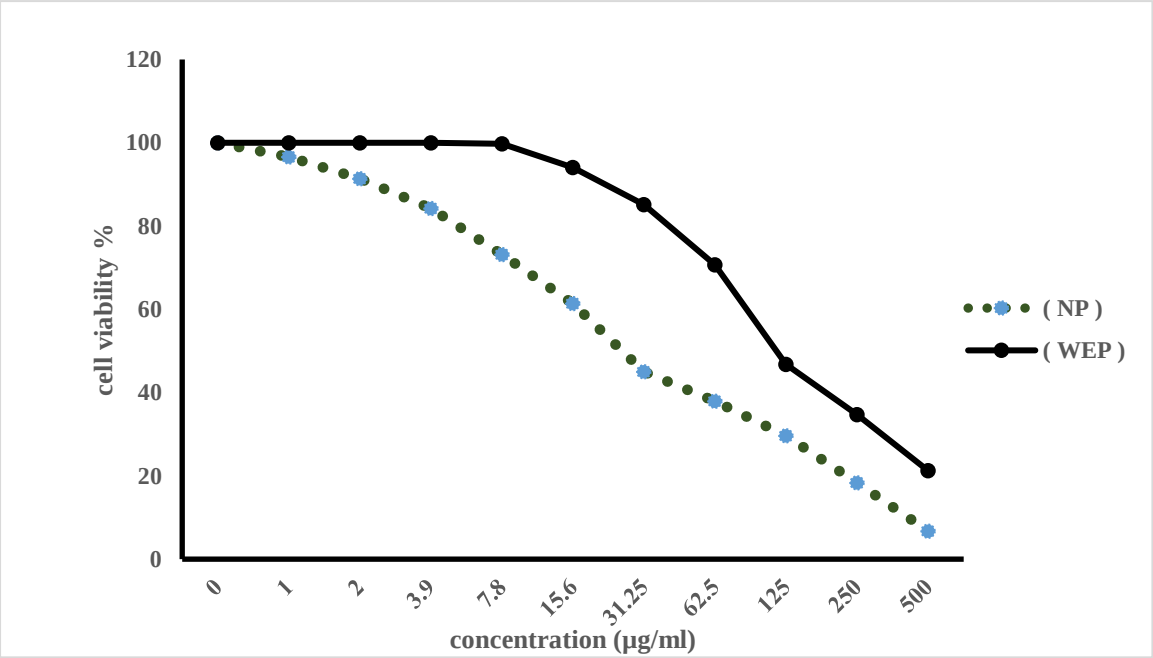

Fig. 7: Inhibitory activity of NP and WEP against Pancreatic carcinoma cells using MTT assay

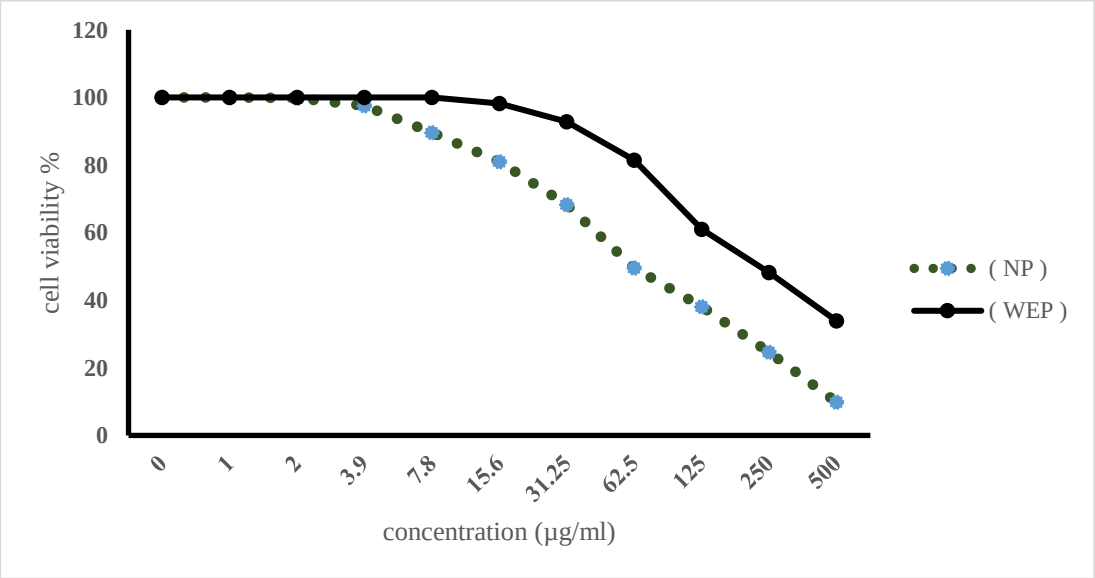

Fig. 8: Inhibitory activity of NP and WEP against MRC-5 using MTT assay

TRAIL-R1, and R-2, which are located on the cell's surface and communicate with the cell's immune system (TNF-related apoptosis-inducing ligand-R1 and R2). Mitochondria and pro-apoptotic proteins, as cytochrome c mediate the second route (intrinsic) [35]. One possible method by which Propolis exerts its therapeutic benefits is activating apoptosis [37]. Propolis's capacity to (1) promote cell death, (2) cause cell cycle arrest, (3) impede cell growth, and (4) cause DNA damage is generally credited for these effects [38]. Researchers looked at whether cisplatin's hazardous side effects may be lessened by administering propolis or chitosan-coated nano-propolis (NP) (CP). Producing NP involves a sustainable sonochemical procedure. When given at 30mg/kg, NP was more efficient than Propolis at mitigating the deleterious consequences of CP (anorexia, weight loss, oxidative damage, and apoptosis) (liver and kidney damage) [39] .

These data suggest that the improved therapeutic efficacy of Propolis and enhanced cellular absorption may be attributable to Nanoparticles of NP and its antioxidant content, which accounts for the difference in IC_{50} values of NP and WEP on

4. Conclusion

From the obvious results, it could be concluded that he nano-propolis (NP) contained the highest addition, their cytotoxicity towards PANC-1 and amounts of total phenolic, total flavonoids, and antioxidant activity than the water extract of propolis (WEP). The polyphenols content of NP and WEP by HPLC found that the NP has high concentrations of Gallic acid, Chlorogenic acid, Taxifolin, and Ellagic acid respectively, as well as; WEP has high concentrations of Cinnamic acid, Kaempferol, Chlorogenic acid, and Pyro catechol, respectively. Moreover, evaluating the antioxidant properties of NP and WEP on MRC-5 cells found that the NP has an IC50 higher than WEP on PANC-1 and MRC-5 as a chemotherapeutic medication. Therefore, propolis' effectiveness was enhanced by being transformed into Nanoparticles to confirm the efficacy of NP in-vitro and in-vivo against certain chemotherapeutic medicines for pancreatic cancer cells.

5. Conflicts of interest

There are no conflicts to declare.

6. Acknowledgment

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