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Chemical Characterization of Pollen Grains Ethanolic Extract (PGE) with Evaluation of its Potent Antioxidant, Antibacterial and Anti-Cancer Activities against Hepatocellular Carcinoma (HepG2)

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ABSTRACT

Background: Pollen grains (PGE) contain a mixture of essential nutrients. Many studies have indicated that polyphenol compounds and, especially, flavonoids are among the 2[ry] metabolites that have many biological activities, such as antioxidant, anticancer and antibacterial activities.

Methods: The main aim of the study is to evaluate the potent and high antioxidant regarding its capability of free radical scavenging activities via ABTS, ORAC and metal chelation assays; anticancer [anti-hepatocellular carcinoma (HepG-2)] and antibacterial activities against the two bacterial strains (*Bacillus subtilis* and *Escherichia coli*) of the pollen grain extract (PGE). Methods: Pollen grain extract (PGE) was chemically characterized using GC-MS, SEM and TEM and antioxidant capacities were determined *via* three assays (ORAC, ABTS and metal chelation) for the estimation of free radical scavenging capacities. antibacterial screening against two bacterial strains (*B. subtilis* and *E. coli*), *in vitro* estimation of cytotoxicity against proliferation of hepatocellular carcinoma (HepG-2).

Result: GC-MS indicated the chemical structure of the pollen grains that contain a lot of phenolics and flavonoids. SEM examination, which revealed the external surface of the used PGE, showed a branched chain appearance with an amorphous structure in a small size. TEM examination, which revealed the internal structure of the used PGE, which appeared as spherical black spots inside a large line-dashed sphere, also clarified that the PGE was found to possess a high potency against the hepatic cancer cells, as evidenced by the inhibition of HepG-2 proliferation growth of cancer cells, which was measured at 84.09 µg/ml at 100 µg/ml. Strong antibacterial capacities of PGE are shown against two strains (*B. subtilis* and *E. coli*), at nearly low concentrations recording growth concentration at 0.625 mg/ml. PGE exhibited a potent antioxidant capacity via recording ABTS and ORAC activities at 1028.76 and 406.87 (µM Trolox eq/ml), in addition to the metal chelation activity by 35.68 (µM EDTA eq/mg extract). Therefore, it can be concluded that the PGE is effective with novel potent antioxidant, antibacterial and anticancer capacities.

Key words: Antibacterial, Anticancer, Antioxidant, Hepatic cancer, Metal chelation, Pollen grains.

INTRODUCTION

Recent studies have looked into the traditional uses of medicinal plants in an effort to validate their use, validate their activities and encourage the pharmaceutical industry to develop new, safe and efficient substitutes (Sadeq *et al*., 2021). Nowadays, a significant portion of the drug substances under study are either natural products or compounds derived from natural sources (Amaral *et al*., 2019). The use of natural products or bio-inspired molecules addresses a variety of illnesses that are pertinent to current public health issues, such as oxidative stress and bacteria resistant to antibiotics.

Antibiotic resistance has become the largest public health concern in recent years. This phenomenon worsens the effects of microbial infections on public health and is brought on by the excessive use of antibacterial medications (Jastaniah *et al*., 2024). Numerous scientists have been drawn to the pursuit of alternative therapeutic approaches through the discovery of additional antibacterial agents (Bakour *et al*., 2021).

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Because of their abundance of bioactive compounds and pharmacological effects, research on beehive products-particularly honey and bee pollen-has garnered a lot of attention lately (Bakour *et al*., 2017). On the contrary, honey bees depend on pollen. They are the primary source of the vitamins, minerals, fats and proteins needed for their diet. The kind of flowers that bees forage on has a significant impact on the composition of honey (Fihri *et al*., 2016).

A variety of vital nutrients found in pollens are utilized by plants to promote growth and development (Alici *et al*., 2014). Therefore, pollen's chemical makeup is mostly dictated by the type of plant that produces it. Various biological activities, including anti-tumoral, anti-diabetic and antimicrobial effects, may be exhibited by their chemical compounds (Mohamed *et al*., 2018) . Numerous studies show that secondary metabolites with a wide range of biological activities, including antibacterial, anticancer and antioxidant, include polyphenol compounds and flavonoids in particular (Arslan *et al*., 2017).

Since the groundbreaking discovery of penicillin, humanity has made progress in the fight against bacteria and other microscopic germs (El-Megharbel and Hamza, 2022, El-Megharbel *et al*., 2025, AlZahrani *et al*., 2025 and Mira *et al*., 2025). They have, however, evolved a resistance to the widely used antibiotics over time in terms of survival and evolution (Yelin and Kishony, 2018). According to WHO, resistant to antibiotics are now posing a major threaten to the general health, which is a concerning situation (Newman and Cragg, 2016) The use of the natural products are being studied as possible antimicrobial agents that could solve the current antibiotic resistance (Cock *et al*., 2017). Reactive oxygen species (ROS) and other human health concerns have also been studied in relation to natural products.

Due to its association with a number of complications, including lung and cardiovascular disorders, some types of cancer, immunological disorders and inflammation, this term has gained popularity recently (Thomas, 1998). ROS is known to have both positive and negative effects on biological processes. The beneficial effects of reactive oxygen species are demonstrated by the way it affects the physiological processes of various cellular reactions. Meanwhile, high levels of the free radicals can harm lipids, proteins and nucleic acids, among other cellular constituents (Cardenas-Rodriguez *et al*., 2013). Over the years, a number of artificial antioxidants have been suggested for the treatment and prevention of specific illnesses; however, their toxicity has resulted in negative consequences when used (Tabti *et al*., 2014).

In this context of recent interest in natural products as an effective solution to recent illnesses and recent antibiotic resistance, pollen grains antibacterial activity was investigated. Specifically, the pollen of the plants has various nutritional and medicinal properties (Sadeq *et al*., 2021). Pollen grain parts are used previously as remedies for the treatment of different diseases such as respiratory infections, nephropathy, high blood pressure and cancer (Baliga *et al*., 2011). It possesses many pharmacological properties such as antioxidant, antifungal and anticancer activities. Additionally, it has the ability to scavenge free radicals and different oxidation in the rat tissue homogenates (Ishurd *et al*., 2005). Moreover, (Ishurd *et al*., 2005) have shown that the glucans prepared from date palm fruits with its contents of pollen manifested a potent anticancer activity. On contrary, *in vivo* study clarified that both aqueous and ethanolic nature of pollen grains elevated the gastrointestinal transit time (Al-Qarawi *et al*., 2003). It also possesses antifungal activity against some fungi such as Candida albicans (Al-Ali and Al-Judaibi, 2019).

Honeybee pollen is considered as a natural food in healthy human diet in many European and Asian countries. The nutritional value of pollen is perfectly balanced. Honeybee-collected pollen typically contains high levels of minerals, appropriate amounts of fat, essential amino acids and 40% proteins. Plant fertilization depends on pollen, the male seed of flowers. All of the necessary elements for life are found in pollen. In addition to proteins, it contains a number of vitamins, hormones, carbohydrates and enzymes or coenzymes. Furthermore, although pollen contains very few calories, it is a rich source of minerals such as magnesium, calcium, copper, manganese and so forth. Pollen has higher levels of proteins, iron, thiamine, riboflavin and niacin than other vegetables and nutrients of the same weight when compared to agricultural crops (Al-Yousef *et al*., 2020).

Products from honey bee like pollen, honey, royal jelly are considered to be promising sources of antioxidants as they are rich in flavonoids like quercetin, kaempferol, naringenin as well as phenolic compounds, including derivatives of cinnamic and benzoic acid (Kalaycıoğlu *et al*., 2017). Pollen, also referred as "the life-giving dust", is an established high-energy food. Extensive research on the properties of pollen extracts from different species has revealed the presence of various phytochemicals including phenols, polyphenols, flavonol and phenylpropanoids, which may have been responsible for their antioxidant properties (Hamza *et al*., 2022).

Thus, this experimental research aims to evaluate the antibacterial activities of the pollen grains extract (PGE) against the two bacterial strains (*B.subtilis* and *E.coli*), anti-hepatocellular carcinoma (HepG-2) and its potent antioxidant activity regarding its ability of free radicals scavenging activities *via* (ABTS, ORAC and metal chelation assays).

MATERIALS AND METHODS

Chemicals

Pollen grains (Organic grains) were obtained from approved natural product (First Elite 100% natural pollen) was obtained from local market company "Wadi A Nahil", KSA. Ethanol and all chemical used were obtained from the "Sigma-Aldrich" Company with highly and pure graded Chemicals.

Extraction

The extraction of pollen grains involved treating 250 g of pollen grains with analytical HPLC-grade 99% ethanol for 24 h at room temperature. The stirring with ethanol was continued for many cycles until exhaustion. The resulting solution was subjected to evaporation on a rotary evaporator at 40°C under reduced pressure to obtain a dark brownish-yellow sticky extract. After that, it was kept at 25°C for about 30 minutes in an ultrasonic bath. After filtering the pollen grain mixture, the extract was gathered and the yellow, sticky residue was preserved, as seen in Fig 1.

GC–MS analysis of pollen grains extract (PGE)

The study used gas chromatography, mass spectrometry and an auto sampler system to analyze PGE. A fused silica capillary column was used for separation and an electron impact ionization system was used as an MS detector. The oven temperature was set to 60°C for five minutes, then 290°C for five minutes. The auto-sampler was used to inject 1 µL of PGE based on the method of analysis described in (Hagag *et al*., 2014).

Cell culture

HepG-2 hepatic cancer cells were procured from Cairo, Egypt. Cells were maintained in DMEM media supplemented with 100 mg/mL of streptomycin, 10 units/mL of penicillin and 10% of heat-inactivated fetal bovine serum in humidified, 5%(v/v) CO_2 atmosphere at 37°C. (El-Megharbel *et al*., 2024).

Cytotoxic activity (IC50 estimation)

The study involved seeding HepG2 cells in 96-well plates and incubating them with a serial dilution of PGE for 24 hours. Cell viability was assessed by SRB assay. Aliquots of 100 µL cell suspension (5×10^3cells) were in the tested well plates and incubated in the media for 24 hr. Cells were treated with other 100 µL media containing PGE at a lot of concentrations. After 72 hr of exposure,the absorbance was measured at 540 nm using a BMGLABTECH®- FLUOstar Omega micro plate reader (Ortenberg, Germany) (Al-Thubaiti *et al*., 2022).

Preparation of inoculum (colony Suspension Method) for *Bacillus subtilis* and *Escherichia coli*

Each *of B.subtilis (ATCC 6633)* and *E.coli (ATCC 8739)* was inoculated into 100 ml of the soy (tryptic) broth medium and then gently incubated at ~35.0°C ± 1.0 for 24.0 hr. For the preparation of the fresh colonies (18-24 hr.) culture agar plates, a loopful from broth was streaked onto (tryptic soy agar) medium and then incubated at the ~35.0°C±1.0.

A sterile saline solution (0.9% NaCl) was prepared by inoculating 3 to 4 colonies and the suspension was adjusted to achieve a turbidity equivalent to a 0.5 McFarland standard of each strain using a DensiCHEK© optical device. That adjustment results in a suspension containing ~1-2 × 108 CFU/ml.

Broth microdilution method

250 µL from the inoculum directly was inoculated in the 1st well in the tested 96 well plate. 100 µL from Muller Hinton Broth media was then gently inoculated in the remaining of the 96 wells. That should result in a final concentration of 5.0×10^5 CFU/mL.

A control well containing inoculated broth media, without the sample. All plates were incubated at ~35.0± 1.0°C for 24.0±2.0 hr (Arnao *et al*., 2001).

Evaluation of the potent antibacterial Activity of PGE against *Bacillus Subtilis* (ATCC 6633) and *Escherichia coli* (ATCC 8739)

After incubation, plates were removed from incubator and placed on dark surface to check growth. All growth control wells yielded turbid solution of growth indicating validity of test.

Control wells were clear and free of turbidity, while all growth wells produced turbid growth.

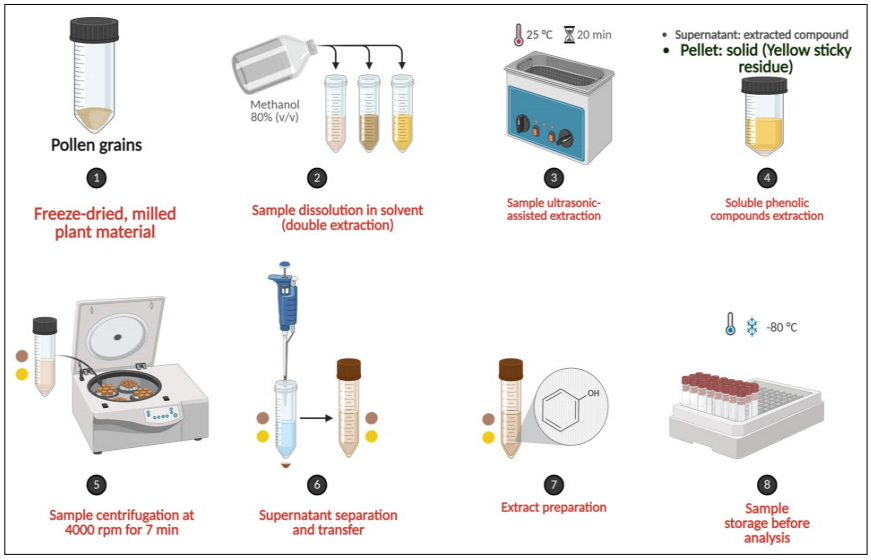

Fig 1: Experimental design for pollen grain extraction procedures.

Antioxidant activity assays

Assay of ABTS

As part of the assay, 192 mg of ABTS were currently dissolved in dist. H_2O, put into a 50 mL flask and then 17 µL of 140 mM potassium persulphate was mixed with 1 mL of the solution. A 96-well plate that containing 10 µL of the sample or compound was combined with the ABTS reagent, the intensity was measured at 734 nm (Santos *et al*., 2017).

Metal chelation

The assay involved a mixture of ferrous sulphate, sample/ compound and ferrozine, incubated at 25°C for nearly 10 minutes. The intensity was measured at ~562 nm as per (Santos *et al*., 2017).

ORAC activity

The study examined the antioxidant activity of PGE and its metal complexes using Liang *et al*.'s method (Liang *et al*., 2014). The fluorescence measurements were conducted for 60 minutes.

SEM and TEM examination

Quanta FEG 250 scanning (SEM) and transmission (TEM) electron microscopes with a 20 kV accelerating voltage were used to visualize the surface morphologies of PGE particles. JEOL JEM-1200 EX II and JEOL 100s microscopy were used to visualize the sizes and shapes of these particles, respectively.

Statistical analysis

The data were presented as mean ± S.E. and the means of the treated groups were compared using a One-Way Analysis of Variance with post-hoc testing. Statistical significance at the significance level $P \leq 0.05$ (IBM, 2020).

RESULTS AND DISCUSSION

Main compound names appeared in GC-Ms with some chemical formula

A total of 12 major compounds identified in GC–MS in PGE extract as follows: Cyclooctasiloxane, 3-Chloropropionic acid, Cyclononasiloxane, n-Hexadecanoic acid, Octadecanoic acid, Tributyl acetylcitrate, Oxiraneoctanoic acid, Oxiraneoctanoic acid, 3-octyl-, methyl ester, Oxiraneoctanoic acid, 3-Octyl-,Octadecanoic acid, Diisooctyl phthalate, Oleic acid as shown in Table 1 and Fig 2.

Anti- hepatocellular carcinoma (HepG2) activity

The study used MasterPlex 2010 software to estimate the half cellular inhibitory concentration (IC50) of HepG-2 cells' *via*bility activity. Results showed that PGE inhibited the growth of HePG2 cells at both 10 ug/mL and 100 ug/mL concentrations, demonstrating its anti-hepatocellular carcinoma capabilities ($P<0.001$) (Fig 3).

Antibacterial Activity Evaluation and MIC reading results

The target pollen grain extract assessed biologically using bacterial strains *B. subtilis* (ATCC 6633) and *E. coli* (ATCC 8739). The antimicrobial activities' results of the pollen grain extract are shown in (Fig 4). It was discovered that pollen grain extract manifested high antimicrobial activities against different bacterial strains and it was sufficient. It was discovered that the tested extract had high levels of inhibition against both bacterial strains at low concentrations of 0.625 mgml for *B. subtilis* and *E. coli* as shown in Table 2 and Fig 4.

Antioxidant activity

The antioxidant capacities which are the main physiological mechanisms in assessment of *invitro* antioxidant capacities of PGE obtained by measuring (ORAC) absorbance, metal chelation and ABTS assay, all are shown in Table 3. Activity of PGE recorded potent antioxidant capacities in chelation of the free radicals and thus exert high antioxidant capacities, this result is of great importance and promising and confirm the high ORAC value of PGE. Table 2. showed that ABTS capacities for PGE is equivalent 406.87 µM trolox capacities, Also, the metal chelation capacities of PGE to scavenge the free radicals is 35.68 ìM EDTA equivalent. Meanwhile, the ORAC capacities showed 1028.76 trolox equivalent capacities.

Trolox eq: Trolox equivalents; EDTA: Ethylene Di-amine tetra acetic acid; SD: The standard de*via*tion; Superscript.

Table 1: Chemical constituents of pollen grains extract (PGE).

RT	Area %	Compound name	Molecular formula	Molecular weight	Cas#
15.90	0.63	Cyclooctasiloxane	$C_{16}H_{48}O_8Si_8$	592	556-68-3
16.40	0.92	3-Chloropropionic acid	$C_{20}H_{39}ClO_2$	346	NA
18.73	1.56	Cyclononasiloxane	$C_{18}H_{54}O_9Si_9$	666	556-71-8
21.18	10.53	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	57-10-3
23.41	6.78	Octadecanoic acid	$C_{19}H_{38}O_2$	298	112-61-8
25.60	1.62	Tributyl acetylcitrate	$C_{20}H_{34}O_8$	402	77-90-7
25.74	0.83	Oxiraneoctanoic acid	$C_{18}H_{34}O_3$	298	24560-98-3
25.94	31.52	Oxiraneoctanoic acid, 3-octyl-, methyl ester	$C_{19}H_{36}O_3$	312	2500-59-6
26.15	0.81	Oxiraneoctanoic acid, 3-Octyl-	$C_{19}H_{36}O_3$	312	6084-76-0
28.44	4.91	Octadecanoic acid	$C_{19}H_{38}O_4$	330	1115-01-1
29.33	1.90	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390	131-20-4
35.05	3.48	Oleic acid	$C_{38}H_{74}O_2$	562	22393-88-0

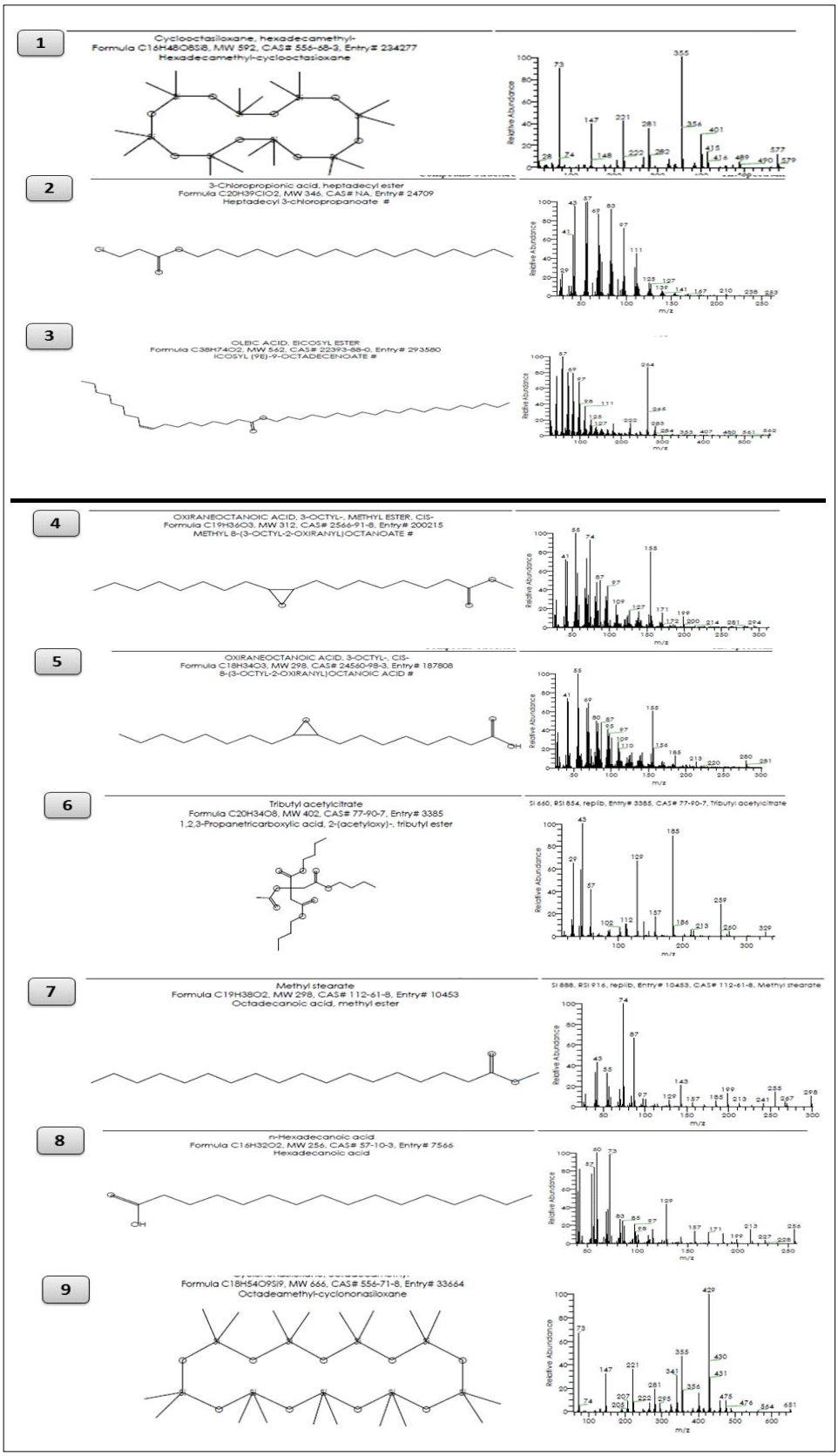

Fig 2: Chemical compound formula of the main components of PGE.

Letters indicate the clear significant differences ($p < 0.05$) ascending alphabetically based on means ranking.

*:Significant difference (P<0.05) ,**:Significant difference (P<0.01), ***:Significant difference (P<0.001).

SEM and TEM

SEM images act as a simple tool technique that is used to illustrate the microscopic and physical character of PGE Fig 5 SEM examination show branched chains appearance with amorphous structure in small size (Fig 5 A1 and A2).

TEM images for PGE is described in (Fig 5 B1 and B2). A spherical black spots inside large line dashed sphere are appeared in PGE.

The real recent inability to treat many diseases due to the high and continuous case of drug-resistant microbes poses a serious and real challenge for global human health. Recently, the development and synthesis of a lot of natural therapeutic active compounds are attracting high attention from the researchers worldwide. Honeybee pollen is considered one of the important natural foods in a healthy human diet; it is a rich source of fats, essential amino acids, carbohydrates, vitamins and minerals (Sultan *et al*., 2023).

What is curious about this result is that PGE contains many important derivatives and essential compounds that have been shown to have antibacterial, antioxidant and even anticancer properties against a variety of cancer types. Cyclooctasiloxane and hexadecamethyloctasiloxane are two of its most crucial ingredients; they have been shown to possess antimicrobial qualities (Khosravani *et al*., 2020) and hexamethylcyclotrisiloxane has been shown to have anticancer properties (Vasudevan *et al*., 2012).

The current findings confirmed presence of important oily component (n-hexadecanoic) , as proven that the first stages of inflammation are the hydrolysis of ester bonds in membrane phospholipids and the subsequent release of fatty acids. Previous studies have been conducted to determine the mechanism by which n-hexadecanoic acid inhibits phospholipase. The enzyme kinetics analysis demonstrated that n-hexadecanoic acid competitively inhibits phospholipase. This suggests that n-hexadecanoic acid, a fatty acid, is an anti-inflammatory substance because it inhibits phospholipase A2. The current study's conclusions support the traditional medical system's strict use of medicated oils high in n-hexadecanoic acid to treat rheumatic symptoms (Agoramoorthy *et al*., 2007).

Since palmitic acid ($CH_3(CH_2)_{14}COOH$) and n-hexadecanoic acid, a saturated fatty acid, are both known to have antibacterial and antifungal properties, the current study found that PGE has strong antibacterial strain's capacities against the two strains (*B. subtilis and E. coli*) at extremely low concentrations of 0.625 mg/ml (Lawrence *et al*.,1993). Additionally, by directly influencing T cells, these essential fatty acids have the ability to alter immune responses (Yu *et al*., 2002). By reducing the synthesis of inflammatory mediators like prostaglandin E2, IL-6, IL-1b, TNF-α and

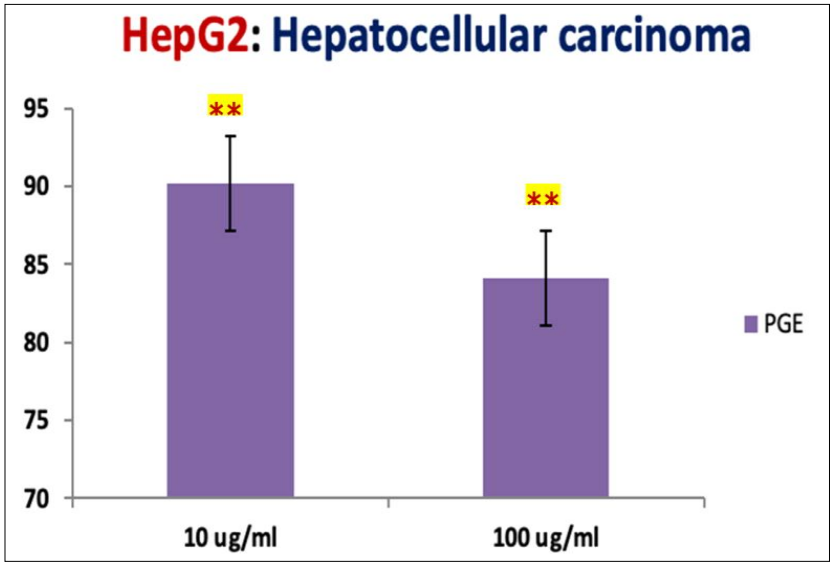

Fig 3: Efficacy of PGE on HepG2 cells *in vitro.*

Table 2: MIC reading results.

Dilution (mg/ml)	5	2.5	1.25	0.625	0.312	0.156	0.078	0.039	0.019
Bacillus subtilis	C	C	C	C	T	T	T	T	T
Escherichia coli	C	C	C	C	T	T	T	T	T

(T; Turbid (-ve results), C; Clear (+ve results).

nitric oxide, conjugated linoleic acid found in food has an anti-inflammatory effect (Hanène *et al*., 2024).

Oleic acid, the main ingredient in olive oil, is another crucial part of the prepared PGE. It has qualities that help lower cholesterol and prevent Alzheimer's and cancer. However, a number of oleic acid derivatives have their own anti-inflammatory and antioxidant properties and they are now being considered as a powerful treatment for obesity. This emphasizes the health advantages of oleic acid. Recent studies indicate that it might affect immune system modulation, particularly through controlling cells involved in the development of inflammation and epigenetic mechanisms (direct changes of DNA and DNA-associated proteins) (Al-Snafi *et al*., 2020).

Recent research has demonstrated the impact of oleic acid (OA) on human health and illness. In addition to its potential to promote wound healing, oleic acid is thought to have modulatory effects on a variety of physiological processes. Some research also indicates that it may have positive effects on autoimmune, inflammatory and cancer conditions. Based on this fatty acid, which is mostly present in the Mediterranean diet, it may play a part in the synthesis of novel therapeutic strategies regarding future for infections, inflammatory, immunological, cardiovascular, or skin repair conditions (Bozorgi *et al*., 2021).

Oxiraneoctanoic acid, a significant derivative found in trace amounts, is one unexpected finding about these results that may provide the additional health benefits, enhancing the functional qualities (Chauhan and Chauhan, 2020).

According to earlier research (Barnawi *et al*., 2023), oleic acid is found in the plasma membrane, whereas palmitic acid and oxiraneoctanoic acid are found mainly in the mitochondria. This may have an impact on the biological effects of these acids. With the exception of oxiraneoctanoic acid, the majority of compounds inhibited CYP1A2, suggesting significant hepatic metabolism. None of them inhibited the activities of either CYP3A4 or CYP2D6, which declines the possibility of drug-drug interactions. These results significantly supported the findings about the effectiveness of synthetic PGE in inhibiting hepatocellular carcinoma (HePG-2).

The most intriguing aspect is that the present study's findings are consistent with earlier research, including one by (Abdel-Rasheed *et al*., 2017), which demonstrated that areca nuts extract, which contains the majority of the abundant fatty acids found in PGE, demonstrated potent anti-aging properties *via* inhibition of the β-amyloid plaques formation (Abdel-Rasheed *et al*., 2017).

While oxiraneoctanoic acid did not exhibit any of these effects, toxicity studies showed that oleic, palmitic and stearic acids can cause skin irritation, eye corrosion and some mitochondrial toxicity. The highest binding affinity was exhibited by 3-octyl oxiraneoctanoic acid, while all other compounds demonstrated moderate binding to plasma proteins. Oxiraneoctanoic acid was categorized as moderately toxic (Class III), whereas oleic, palmitic and stearic acids were categorized as low toxicity (Class IV) (Al-Snafi, 2020).

The current study revealed potent and high antibacterial activity of the PGE and these findings are in complete accordance with the previous study of Barnawi *et al*., 2023, who confirmed that pollen grain extract exhibited antibacterial activity with more inhibition zones in comparison to the maize grain extract, which exhibited a fewer inhibition zone against some organisms, such as *E. coli* and *C. albicans*. However, contrary to the current results, as the used PGE exhibited antibacterial activity against B. subtilis, meanwhile, Barnawi *et al*., 2023 revealed that *B. subtilis* was high sensitive than the maize pollen grain extract than the clover pollen grain extract.

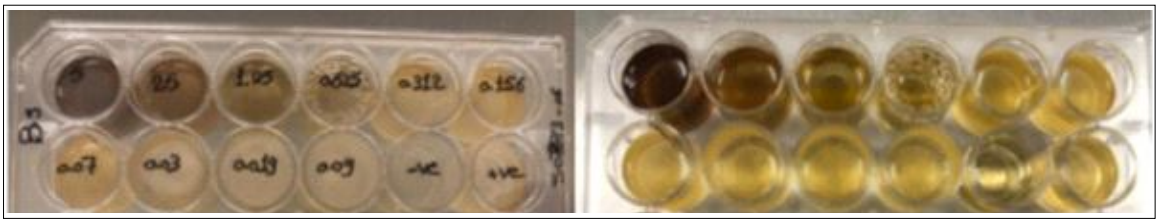

Fig 4: Bacterial growth for PGE on *B.subtilis* (ATCC 6633) strain.

Table 3: Antioxidant capacity of pollen grains extract (PGE).

Sample name	ABTS (µM trolox eq/mg)		ORAC (µM Trolox eq/mg extract)		Metal chelation (µM EDTA eq/mg extract)	
	Mean	SD	Mean	SD	Mean	SD
PGE	406.87^{b**}	40.68	1028.76^{a***}	92.98	35.68^{c***}	5.98

Trolox eq: Trolox equivalents; EDTA: Ethylene Di-amine tetra acetic acid; SD: The standard deviation; Superscript.

Letters indicate the clear significant differences ($p<0.05$) ascending alphabetically based on means ranking.

*: Significant difference ($P<0.05$) ,**: Significant difference ($P<0.01$).

***: Significant difference ($P<0.001$).

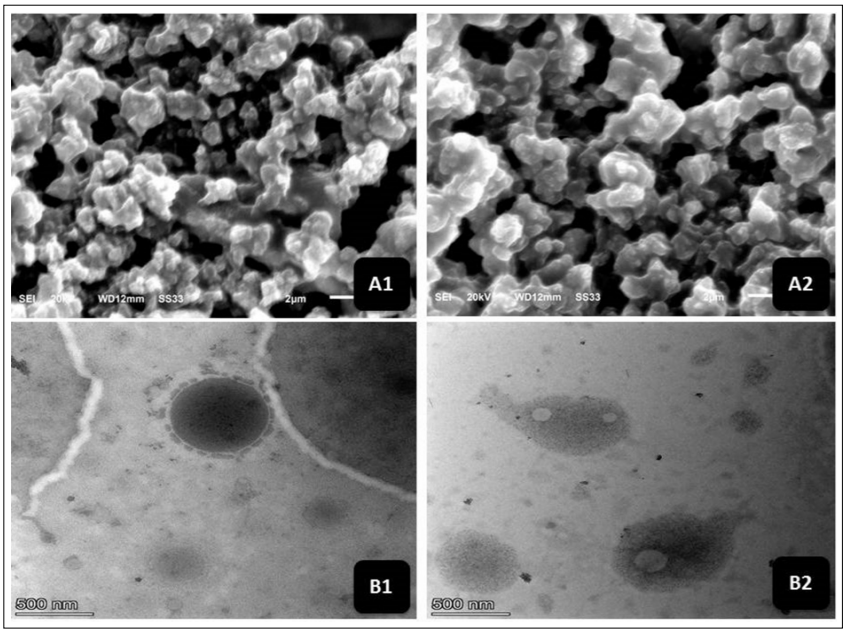

Fig 5: SEM (A1,A2) (500 nm) TEM (B1,B2) (2 µm) of pollen grains.

Results of the present study were in high agreement with previous reports, where different extracts of pollen grains exhibited higher diameter of inhibition zones than some other extracts of maize against Paenibacillus larvae (Abdel-Rasheed *et al*., 2017).

In addition to the antibacterial activity of the used PGE which may be attributed to its constituents from quercetin and kaempferol (contents of pollen grains extract) with different concentration values.

In the present study, PGE possessed a high content of hexadecanoic acid besides many flavonoids. The presence of these flavonoids showed a significant antioxidant activity of pollen grains. Leja *et al*., 2007 reported that the phenolics, flavonoids and pigments such as β-carotene were responsible for the mechanism of actions of antioxidant capacitie s of the pollen grains. Mãrghita *et al*., 2009 mentioned that these active compounds that appeared in the structure of PGE played a very vital role in prevention of the oxidative injury of some cellular biomolecules.

The strong antitumor potential of fatty acids, such as oleic acid, palmitic acid, stearic acid and oxiraneoctanoic acid, is highlighted by the cytotoxicity data from a prior study (Abdel-Rasheed *et al*., 2017). Broad-spectrum antitumor activity was demonstrated by oleic acid's notable cytotoxic effects on a variety of cancer cell lines, particularly against melanoma and non-small cell lung cancer. Oxiraneoctanoic acid, on the other hand, stood out for its strong anti-leukemia (H9) properties, as well as its notable anti-lung cancer and special potent effects on the hematopoietic tumors. It is a *via*ble option for targeted anticancer treatments because of its specificity.

The current study was in accordance with (Barnawi *et al*., 2023), who proved the anticancer activity of two extract types of the pollen grains against the colon cancer cell line (HCT-116). These alterations in the treated cells with pollen grain extracts have documented many antitumor markers' capacities of PGE against cancer cells, as proven in the present study.

Thus, the current results greatly confirmed the high antioxidant, antibacterial and anti-hepatocellular carcinoma (HepG-2) *via* the chemical characterization and biological assays that confirmed this concept in accordance with previous literatures and thus, clinical studies are recommended and more prospective studies regarding inorganic metal drug complexation with PGE to evolve more novel data regarding this vital compound.

CONCLUSION

PGE was characterized after chemical extraction and characterization by using GC-Ms, SEM and TEM. For PGE biological potencies, Antioxidant capacities as Trolox equivalent was compared using ORAC, ABTS and Metal chelation assays. PGE induced high potent antioxidant capacities, PGE mainfested a high antioxidant capacity *via* recording ABTS and ORAC activities at 1028.76 and 406.87 (µM Trolox eq/ml), in addition to the metal chelation activity by 35.68 (µM EDTA eq/mg extract), strong antibacterial properties of PGE are shown against the two strains (*B.subtilis* and *E.coli*), at very low concentrations recording growth concentration at 0.625 mg/ml, with enhanced the anticancer activities by inhibition of the hepatocellular carcinoma (HepG-2) proliferation growth by 90.16 and 84.09 µg/ml. Thus, used PGE possessed antioxidant, antibacterial and anticancer activities.

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Conflict of interest

All authors declare that they no conflict of interest.

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