



ANTICANCER POTENTIAL OF POLYPHENOLIC
COMPOUNDS OF BARHI DATE PALM KERNELS
THROUGH INDUCTION OF APOPTOSIS IN CANCER
CELLS.

BY

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ABSTRACT

Cancer is a major burden of disease worldwide, with an estimated 6.9 million cancer deaths in 2018. The number of new cases is expected to increase by about 70% over the next two decades. Polyphenolic compounds (extractable, EPPs and non-extractable, NEPPs), which are most abundantly present as natural bioactive compounds, have recently shown to be a powerful *in-vitro* anticancer activity through inducing apoptosis in cancer cells. Date palm kernels have gained interest as a valuable by-product “waste” of the date fruit industry and have been identified as a rich source of EPP and NEPP. Therefore, this study aimed to investigate the anticancer potentiality of polyphenols from Barhi date palm kernels (BDPK) extracts through inducing apoptosis in cancer cells. In the first stage of the study, chemical composition of BDPK was performed before the extraction process to identify its nutritional quality. Cytotoxic and antioxidant activities were screened in aqueous and organic extracts of BDPK by using *in-vitro* assays, and accordingly, the optimisation of a set of extraction conditions for high-yield recovery of EPP and NEPP, with high anticancer and antioxidant activities, was performed using response surface methodology (RSM). From the obtained results, it can be revealed that EPP exhibited more promising cytotoxic and antioxidant properties when compared to NEPP. Therefore, screening phenolic profile by LC-QTOF/MS to identify new cytotoxic compounds in the active EPP crude extract was done in the next stage. The isolation process was performed through successive steps of various chromatographic methods which had been assisted by the cytotoxic and antioxidant activities of the separated fractions and sub-fractions. Three new cytotoxic flavonoids nobiletin (**C1**, NOB), tectorigenin (**C2**, TEC) and persicogenin (**C3**; PERS) were successfully isolated and identified from BDPK for the first time. The final structures of the isolated flavonoids were established with the aid of spectroscopic analysis including one- and two-dimension at nuclear magnetic resonance (NMR), melting points (m.p.), preparative thin layer chromatography (TLC) and mass spectrometry (MS). The three isolated flavonoids exhibited cytotoxic effects against two human cancer cell lines; lung (A549) and colon (HT29), but not normal cells, with higher cell death in A549 than in HT29 after 72 hours treatment, where PERS was found to be the most potent compound, followed by NOB and TEC ($IC_{50}=5.75\mu\text{g/mL}$, $8.56\mu\text{g/mL}$, $10.48\mu\text{g/mL}$, respectively). Subsequently, the mechanism of cell death induced by BDPK crude extracts (EPP and NEPP), and the isolated compounds was demonstrated in the last stage of the research. After performing various morphological, biochemical and molecular assessments, it was established that the BDPK extracts induced late stages of apoptosis as there was evidence of the DNA degradation, and large percent of the cells population being in the sub- G_0 phase induced by the two extracts. Furthermore, EPP and NEPP exhibited dependent mitochondrial signalling pathway as seen with caspase-9 and induced receptor-mediated (extrinsic) apoptotic pathway as seen with caspase-8. In conclusion, the present study has revealed the anticancer potentials of BDPK extracts and the three new cytotoxic isolated compounds may be new promising preventive/therapeutic candidates in treating lung and colon cancers.

خلاصة البحث

يعد السرطان عبئاً مرضياً رئيسياً في جميع أنحاء العالم ، حيث يقدر عدد الوفيات بالسرطان بحوالي 6.9 مليون شخص في عام 2018. ومن المتوقع أن يزداد عدد الحالات الجديدة بنسبة 70٪ خلال العقدين المقبلين. وقد أظهرت المركبات البوليفينولية (القابلة للاستخلاص ، EPPs وغير القابلة للاستخلاص ، NEPPs) ، والتي هي أكثر المركبات الطبيعية توافراً كمواهب بيولوجية فعالة ، أظهرت في الآونة الأخيرة فعالية كبيرة ضد السرطان في المختبر من خلال إحداث الموت المبرمج في الخلايا السرطانية. اكتسبت حبات نخيل التمر الاهتمام باعتبارها منتجات ثانوية أو "نفايات" قيمة من معامل تصنيع فاكهة التمر وقد تم اعتبارها كمصدر غني بال EPP و NEPP. لذلك، هدفت هذه الدراسة إلى تقييم قدرة البوليفينول المستخلصة من حبات تمر البرحي (BDPK) كمضادات للسرطان من خلال إحداث موت الخلايا المبرمج في الخلايا السرطانية. في المرحلة الأولى من الدراسة، تم تحليل التركيب الكيميائي ل BDPK قبل عملية الاستخراج لتحديد جودته التغذوية. تم تقييم فعالية المستخلصات المائية والعضوية من BDPK كمضادات للأوكسدة وقتل الخلايا السرطانية باستخدام فحوصات مخبرية، بعدها تم استعمال عدد من التقنيات الإحصائية بصورة متتابعة لتحسين عملية استخلاص EPP و NEPP، وزيادة فعالية سمية الخلايا السرطانية ومضادات الأوكسدة، وذلك باستخدام منهجية سطح الاستجابة (RSM). أظهرت نتائج الدراسة المسحية أن مستخلص EPP أظهر خصائص كمضاد للسرطان وكمضاد للأوكسدة بفعالية أكبر بالمقارنة مع NEPP. وعلى هذا الأساس، وفي محاولة لعزل مركبات جديدة ذات فعالية سمية للخلايا السرطانية من خلاصة EPP الخام وتحديد هياكلها تمت في المرحلة التالية، تم إجراء فحص البوليفينولات بواسطة LC-QTOF / MS لتحديد مركبات سامة جديدة في مستخلص EPP الخام الفعال. في المرحلة التالية تمت عملية العزل من خلال خطوات متتالية من طرق كروماتوجرافية مختلفة والتي كانت تعتمد على الفعالية السمية للمركبات المعزولة والمركبات الجزئية الناتجة عنها ضد الخلايا السرطانية وكمضادات للأوكسدة. تم بنجاح ولأول مرة عزل وتعريف ثلاثة فلافونويدات سامة للخلايا السرطانية (C1 nobiletin، (C2 و NOB) tectorigenin، (TEC) و persicogenin (C2؛ PERS) من BDPK لأول مرة. تم تحديد التركيبات النهائية للفلافونويدز المعزولة بمساعدة التحليل الطيفي وتشمل استخدام جهاز الرنين المغناطيسي النووي (NMR) و نقطة الانصهار (p.m) وكتلة الطيف الكتلي (MS). أظهرت مركبات الفلافونويد الثلاثة المعزولة تأثيرات سامة للخلايا ضد نوعين من الخلايا السرطانية، وهي سرطان الرئة (A549) وسرطان القولون (HT29)، ولم تتأثر الخلايا الطبيعية. بالمقارنة وُجد ان هذه المركبات ذات كفاءة عالية على قتل الخلايا السرطانية وفعاليتها اقوى على اورام الرئة A549 منها على اورام القولون HT29 بعد 72 ساعة من العلاج، حيث وُجد أن المركب PERS هو الأشد فعالية، يليه المركب NOB واخيراً المركب TEC (IC50 = 5.75µg/mL ، µg/mL 8.56 ، 10.48 µg/mL، على التوالي). بعد ذلك، تم دراسة آلية موت الخلايا الناجم عن مستخلصات BDPK الخام (EPP و NEPP) والمركبات التي تم عزلها في المرحلة الأخيرة من البحث. بعد إجراء العديد من التقييمات الشكلية والكيميائية والجزئية ، ثبت أن مستخلصات BDPK تسببت في إحداث مراحل متأخرة من موت الخلايا المبرمج ، حيث كان هناك دليل على اتلاف الحمض النووي، ونسبة كبيرة من اعداد الخلايا في الطور تحت G₀ بفعل المستخلصين. علاوة على ذلك، وُجد ان المستخلصات EPP و NEPP عملت على تحطيم الغشاء الخلوي للخلايا المعالجة ونشاطية انزيمات الكاسباز 9-caspase والمسار المستقبلي (الخارجي) انزيمات كاسباز 8-caspase. أخيراً، كشفت الدراسة الحالية عن فعالية مستخلصات ال BDPK والمركبات الفعالة الثلاثة الجديدة التي تم عزلها في قتل الخلايا السرطانية والتي من الممكن ان تكون مرشحات وقائية وعلاجية كيميائية واعدة جديدة في علاج سرطان الرئة والقولون.

APPROVAL PAGE

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DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Israa Ali Mahmud

Signature

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In the name of ALLAH the most Merciful and Beneficent

All praise be to Allah,

and peace and blessings be upon His honest Messenger

Mohammed bin Abdullah and upon his family and companions.

First and foremost praise is to ALLAH, the Almighty, the greatest of all, on whom ultimately we depend for sustenance and guidance. I would like to thank Almighty Allah for giving me opportunity, determination, health and strength to do my research. His continuous grace and mercy was with me throughout my life and ever more during the tenure of my research. All praise to Allah Subhanahu Wata'allah for everything HE has given me.

﴿وَمَا تَوْفِيقِي إِلَّا بِاللَّهِ عَلَيْهِ تَوَكَّلْتُ وَإِلَيْهِ أُنِيبُ﴾

[سورة من الآية: 88]

﴿وَاتَّقُوا اللَّهَ وَبِعَلِّمُوهُنَّ اللَّهَ وَاللَّهُ بِكُلِّ شَيْءٍ عَلِيمٌ﴾

[البقرة: 282]

﴿رَبِّ أَوْزَعْنِي أَنْ أَشْكُرَ بِعَمَلِكَ الَّذِي أَنْعَمْتَ عَلَيَّ وَعَلَىٰ وَالِدَيَّ وَأَنْ أَعْمَلَ صَالِحًا

تَرْضَاهُ وَأَدِّبُنِي بِرَحْمَتِكَ فِي عِبَادَتِكَ الصَّالِحِينَ﴾

[البقرة من الآية: 19]

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