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Antioxidant Properties and Total Polyphenolic Contents of Post Distillation Solid Residue Extract from Gamma-Irradiated Clove Buds

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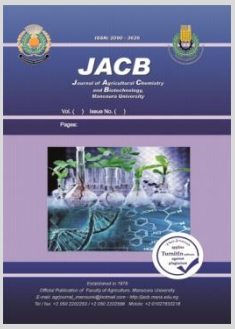

ABSTRACT

The current study was carried out to investigate the antioxidant activities of the solid waste residue obtained after removing the essential oils through the distillation of γ-irradiated clove buds at three levels of doses (0, 5 and 10 kGy). The total phenolic and flavonoid contents (TPC and TFC) were assessed followed by HPLC identification and quantification. The DPPH and FRAP methods were used to measure the antioxidant activity. The obtained results showed that 10 KGy doses greatly improved the TPC, TFC, and antioxidant activities of the solid residue of clove buds. Ten phenolic components and eight flavonoid compounds were detected in non-irradiated and irradiated at 10 kGy in methanolic extracts by HPLC. Various levels of methanolic extract of the solid residue of irradiated clove buds at dose level of 10 kGy (100, 200, and 400 mg/L) were tested for these antioxidant activities against the oxidative changes in sunflower oil compared with control and BHT-treated samples. The sample containing clove solid residue at levels of 400 mg/L showed a good potential for oxidative stability (6.95h). Finally, the data obtained demonstrated that clove extracts are a promising source of bioactive components and a natural supply of antioxidant compounds. Hence, it also offers a variety of possible applications in the food and pharmaceutical industries.

***Keywords*:** Gamma irradiation**;** Clove buds *(Syzygium aromaticum)* **;** Antioxidant activity **;** post-distillation waste residue**;** Oxidative stability.

INTRODUCTION

One of the primary causes of food quality degradation is lipid peroxidation. Lipid peroxidation causes the formation of reactive oxygen species and free radicals, which are purportedly associated with carcinogenesis, mutagenesis, inflammation, DNA changes, aging, and cardiovascular diseases *(*Siddhuraju and Becker 2003 and Shahid *et al.*, 2008).

Synthetic antioxidants like tertbutyl hydroquinone (TBHQ), butylated hydroxyl anisole (BHA) and butylated hydroxy toluene (BHT) can significantly slow down lipid oxidation, but their use as food additives is limited as they may contain numerous factors that are harmful to health and may be linked to carcinogenic, mutagenic, and aging effects (Bopitiya and Madhujith, 2014, Padmaja and Prasad, 2011). As a result, consumers believe that antioxidants from natural resources are superior and safer than the synthetic ones. Therefore, natural antioxidants in foods are being developed to replace the need for synthetic antioxidants in foods (El-Hadary and Taha, 2020).

Aromatic plants and spices are widely utilized in a wide range of food products, including meat and its derivatives, bread, and dairy products. The molecular nature and composition of aromatic oils are crucial factors in determining their antibacterial action. Clove (Syzygium aromaticum, Myrtaceae) is a plant commonly used in the food sector based on its distinct flavor and medicinal uses. (Devkota and Adhikari-Devkota 2020, Bhavaniramya *et al.*, 2019). Clove is a versatile spice for culinary preparation, and their aromatic oils are utilized to flavor food and beverages. Clove oil is abundant in phenols and has strong antioxidant properties (Cortes-Rojas *et al.*, 2014, Shan *et al.*, 2005). According to (Gulcin *et al.*, 2012), Cloves have antioxidant properties similar to α-tocopherol, BHA, and BHT.

Recently, there has been increased interest in utilizing agricultural waste as a low-cost, long-term source of phenolic compounds and their applicability as safe and natural antioxidants (Santana-Me'ridas *et al.*, 2012). Aromatic plants are rich in phenols, terpenes, flavonoids, ascorbic acid, nitrogen compounds, and other antioxidant compounds. Because phenolic compounds are non-volatile and thermally inert, the majority of them remain in the solid left after the extraction of essential oil. Several investigations have indicated solid residues as a viable source of phenolic component recovery. Both Saha and Basak (2020) and Turrini *et al.* (2021) proposed these wastes as prospective sources potentially usable for recovering bioactive phenolic compounds. Additionally, Parejo *et al.* (2002) found that distilled plant material from six aromatic plants has more antioxidants than the undistilled plants. Additionally, Nieto *et al.* (2011) showed the possible viability of adding solid R. Officinalis residues to pregnant ewes' feed as an antioxidant supplement to prevent meat lipid oxidation.

Gamma irradiation has been approved for food preservation by the United States Department of Agriculture (USDA) and the Food and Drug Administration (FDA). According to the FDA (1981), food irradiated with 50 kGy or less is safe for human consumption. γ-irradiation may preserve the antioxidant activity of numerous plant materials.

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According to Behgar *et al.* (2011), radiation can raise or reduce the amounts of antioxidant phytochemicals in plants. Harrison and Were (2007) also discovered that phenolic content in clove, almond skin, and cinnamon increased following gamma irradiation, however phenolic content in nutmeg remained unaltered. In contrast, Ahn *et al.* (2005) reported that low and medium doses have minimal effects on phytochemical antioxidants, with up to 10 kGy not influencing on the antioxidant activity of cinnamon, anise, nutmeg, mint, ginger, licorice, and vanilla. Furthermore, a dose of 10 kGy raises the total phenolic and flavonoid content of mugwort extract (Hwang *et al.*, 2021).

To our knowledge, no research on the influence of gamma radiation on the phytochemical content and antioxidant activity of clove solid residue has been published. This study aimed to evaluate the methanolic extracts of post-distillation waste from γ-irradiated clove buds (*Syzygium aromaticum*) at doses of 0, 5, and 10 kGy as natural antioxidant agents.

MATERIALS AND METHODS

Materials:

Dried clove buds *(Syzygium aromaticum)* were gathered from the Local market in Cairo, Egypt. Folin-Ciocalteu reagent, quercetin, gallic acid, iron (III) chloride, and aluminium chloride, 2,2-Diphenyl-1- picrylhydrazyl (DPPH), Butylated hydroxyl toluene (BHT) were acquired from Sigma (St. Louis, MO, USA). methanol absolute obtained from EL-Gomhouria Trading Chemicals and Drugs Co. Refined sunflower oil and without synthetic antioxidants) was purchased from ARMA for food industry Co., 10th of Ramadan, Cairo, Egypt.

Methods:

Irradiation treatments

Dried clove buds samples were exposed to gamma rays at doses of 0, 5, and 10 kGy using a 60 Co Russian gamma chamber (dose rate 1.3 kGy/h) from the Cyclotron Project, Nuclear Research Center, Atomic Energy Authority, Cairo, Egypt.

Extraction:

One hundred grams of dried clove samples were hydro-distilled for six h using a Clevenger-type apparatus (Senthilkumar *et al.*, 2009). The oils were then dried over anhydrous sodium sulfate to remove any remaining water and kept in sealed vials at a low temperature (4°C) until use (not used in the present study). The waste plant material collected after the essential oils were extracted through distillation (solid waste residue) was dried in an oven at 60 °C for 48 hours before being used for further analysis. The dry byproduct was grounded using an electric blender (Moulinex, France). Approximately 50 grams of dried solid residue were fully extracted with methanol (70%) and mechanically shaken in an orbital shaker for 48 hours at room temperature. The supernatants were then filtered under vacuum (Whatman grade 1) and evaporated in a rotary evaporator to get the dried extracts, which were stored in sealed tubes at 4 °C until analysis.

Total phenolic content:

The total phenolic content was evaluated using the Folin-Ciocalteu method developed by Arabshahi-Delouee and Urooj (2007). The results were given as mg Gallic acid equivalent (GAE)/100 g dry weight (DW)

Total flavonoid content:

The total flavonoid content was measured using the procedure outlined by Ordon *et al.* (2006). Results were represented as mg quercetin equivalent (QE)/100 g dry weight (DW).

Determination of Antioxidant activity:

DPPH method:

The ability of the obtained extracts to donate electrons was assessed by bleaching the purple-colored DPPH solution following the methods of Gulcin *et al.* (2004). The free radical DPPH's antioxidant activity was computed as follows:

$$(1 - [A_{sample} / A_{control}]) \times 100.$$

Where

$(A)_{control}$ is the absorbance of the control and $(A)_{sample}$ is the absorbance of the test extracts.

FRAP method:

The reducing power of each extract was measured by the Oyaizu method (1986).

Fractionation and identification of phenolic and flavonoid compounds by HPLC:

Phenolic and flavonoid compounds were measured in un-irradiated and irradiated samples at 10 kGy using the procedures outlined by Goupy *et al.* (1999) and Skerget *et al.* (2005), respectively.

Rancimat technique measurement of sunflower oxidative stability:

Oil samples were tested for oxidative stability using Rancimat (Metrohm 679) equipment and the Markus and Peter technique (1986). Sunflower oil fortified with methanolic extracts of irradiated clove buds at 10 kGy concentrations (100, 200, and 400 mg/L), then fully blended with a magnetic stirrer to obtain total distribution throughout the oil. Sunflower oil samples were preserved without any additions as a control and BHT (200 mg/L) as a reference antioxidant. The induction period (I.P.) was run at 110 °C using Rancimat and estimated at 25 °C using a temperature coefficient of 2.0 for the expired period. Antioxidant activity and increasing index were measured by the equations described by Khalaf, *et al.* (2018).

$$\text{Antioxidant activity} = \frac{\text{Induction period of sample}}{\text{Induction period of control}} \quad (1)$$

$$\text{Increasing index} = \frac{\text{Induction period of sample} - \text{Induction period of control}}{\text{Induction period of control}} \times 100 \quad (2)$$

Statistical analysis:

Results were treated to one-way ANOVA and Duncan's multiple range tests by the SPSS computer program (SPSS, 2009) and displayed as mean ± SE (n = 3) ($P < 0.05$).

RESULTS AND DISCUSSION

Total phenolic and flavonoid content:

Polyphenols are bioactive plant secondary metabolites found naturally in regularly ingested plant foods. Table 1 shows the TPC and TFC contents of clove solid residue methanolic extracts. In this regard, γ-irradiation increased TPC and TFC. The highest TPC and TFC of clove (1976.5±1.68 mg GAE/100g DW) and (327.17±1.88 mg QE /100g DW, respectively) were detected

at dose 10 kGy. It can be concluded that 10 kGy improved TPC and TFC when compared to the control and 5 kGy. The current results are congruent with those of Aly *et al.* (2016) and Al-Kuraieefa and Alshawia (2020) who found that gamma irradiation (5-15 kGy) increased the phenolic, flavonoid, and antioxidant content of Moringa oleifera and thyme, respectively. In the same concern, El-Beltagi *et al.* (2020) showed that celery seeds and dates fruits have higher phenolic and flavonoid contents at γ-irradiation doses (2.5–10 kGy). Furthermore, Hwang *et al.*(2021) discovered that a 10 kGy dosage improves the total phenolic and flavonoid content of mugwort extract. On the other side, several types of research have shown that gamma irradiation has negative effects. For instance, a dosage of 5 kGy lowered the Cuscuta chinensis L. extract's total phenol and flavonoid levels by 12% and 18%, respectively (Alijaniha *et al.*, 2021). Meanwhile, a dosage of 10 kGy decreased the total phenolic and flavonoid content of the Bondoky and Sakouti dry date (El-Beltagi *et al.*, 2019). The influence of radiation on total phenolic content varies depending on plant type, sample state (solid or dry), geographical and environmental circumstances, temperature, extraction solvent, extraction processes, phenolic content composition, and gamma irradiation dose, etc. (Ibrahim *et al.*, 2016). Gamma radiation can increase the total amount of phenolic compounds present, which may be caused by the breakdown of some insoluble phenolic compounds, the release of phenolic compounds from glycosidic components, or the decomposition of larger phenolic compounds into smaller ones (De Camargo *et al.*, 2012).

Table 1. Effect of gamma irradiation on total phenolics (TPC) and total flavonoid (TFC) contents in methanolic clove solid waste extract (Means ± SE).

γ - irradiation (kGy)	TPC (mg GAE/100 g DW)	TFC (mg QE/100 g DW)
0	1877.17±1.82^{b}	318.01±0.87^{b}
5	1824.65±0.59^{c}	317.72±0.73^{b}
10	1976.5±1.68^{a}	327.17±1.88^{a}

Means with the same letter in the same columns are not significantly different ($p > 0.05$).

DPPH radical-scavenging activity:

The scavenging effect of methanolic clove solid waste extract and BHT on DPPH radicals were compared and expressed as % (Table 2). The obtained data revealed that the antioxidant activity of clove solid residue varied from 83.64 to 90.17% after 30 min in non-irradiated samples. On the other hand, the methanolic extract of clove buds exposed to 10 kGy radiation exhibited much higher antioxidant activity (90.17% after 30 min.) than BHT value (49.44% after 30 min.). As a result, the strong scavenging ability of clove solid residue at 10 kGy can be linked to its high phenolic content. The current results are in line with those of Harrison and Were (2007), who showed that the maximum scavenging capacity of almond skin extracts was related to the higher total phenolics content of irradiated samples at the higher dosage level of 12.7 kGy. Additionally, Khattak *et al.* (2008) discovered that raising the γ-irradiation dose improved the free radical-scavenging action of Nigella sativa seed extracts. Khawory *et al.* (2020) also stated that the leaves of Euodia malayana, Gnetum gnemon, and Khaya senegalensis have higher total phenolic contents and antioxidant activity after exposure to gamma irradiation at levels of 3–13 kGy. The improved antioxidant activity of a plant following irradiation is primarily attributed to greater enzymatic activity (for example, phenylalanine ammonia-lyase and peroxidase activity) or increased tissue extractability due to the depolymerization and dissolving of cell wall polysaccharides (Alothman *et al.*, 2009).

Table 2. The ability of non-irradiated and irradiated clove solid waste extracts to scavenge DPPH radicals

γ - irradiation (kGy)	% Scavenging activity of DPPH zero time	after 30 min
0	83.64±0.3^{b}	88.89±0.54^{b}
5	83.52±0.1^{b}	89.18±0.33^{b}
10	85.43±0.03^{a}	90.17±0.21^{a}
BHT(200ml/L)	27.21±0.22^{C}	49.44±0.07^{C}

Means with the same letter in the same columns are not significantly different ($p > 0.05$).

Ferric reducing antioxidant power (FRAP):

Table 3 shows the FRAP values of the clove solid waste extract. There was a considerable and/or negligible increase in the FRAP of solid waste extract of all irradiated cloves as compared to non-irradiated extract and synthetic antioxidants (BHT). At a gamma irradiated dose of 10 kGy, the methanolic clove solid waste extract exhibited the highest FRAP value (2.73±0.04) followed by 5 kGy (2.60±0.02), non-irradiated (2.49±0.04), and BHT (1.030±0.082**).** The results demonstrated a connection between the FRAP and the phenolic content of the extracts. This demonstrates the reducing power observed in this study, may be based on the high characterized by their powerful electron-donating ability (Bilot *et al.*, 2012).

Table 3. Ferric reducing antioxidant power (O.D) of non-irradiated and irradiated clove solid waste extracts (Means ± SE).

γ -irradiation (kGy)	FRAP (O.D)
0	2.49±0.04^{c}
5	2.60±0.02^{b}
10	2.73±0.04^{a}
BHT(200mg/L)	1.030±0.082^{d}

Means with the same letter in the same columns are not significantly different ($p > 0.05$).

Fractionation and identification of phenolic compounds in methanolic extracts:

The data shown in Fig. (1) revealed the presence of ten phenolic compounds. Eugenol is the most abundant phenolic component in methanolic clove solid waste extracts (33.6 mg/mL), followed by myricetin, protocatchuic, ellagic, syringenic, gallic, caffeic, salicylic, rosmarinic, and cinnamic. The phenolic components in clove solid residue methanolic extracts were significantly affected by the irradiation treatment. A radiation dose of 10 kGy increased the phenolic content. For instance, the concentrations of eugenol, caffeic acid, rosmarinic, gallic acid, Salicylic acid, and ellagic acid were increased from 33.6, 5.66, 2.33, 7.14, 3.12, and 8.36 mg/mL to 35.26, 15.26, 6.14, 8.56, 5.47 and 9.11 mg/mL, respectively. In contrast, Radiation dose 10 kGy reduced the number of certain phenolics; the amount of cinnamic acid, myricetin, syringenic acid, and protocatchuic acid was decreased from 0.89, 11.2, 8.10, and 9.68 mg/mL to 0.78, 7.12, 3.10 and 2.33 mg/mL, respectively. As a result of increased phenolic acids (induced by irradiation at doses of 10 kGy), plant bioactivity is increased, which can have beneficial and

improved effects on human health and greatly strengthen the immune system. The results agree with those of (Variyar *et al.*, 1998) who discovered that a radiation dose of 10 kGy raised the phenolic acid concentration of clove and cinnamon while leaving nutmeg unaffected. Also, Pereira *et al.* (2017) discovered a statistically significant increase in the amount of phenolic and flavonoid components evaluated by HPLC in lemon verbena and peppermint infusion samples exposed to 10 kGy of radiation. on the other hand, Zhu *et al.* (2010) found that dosages ranging from (2 to 10 kGy) significantly reduced three phenolic acids (sinapinic acid, p-coumaric acid, and Ferulic acid).

Radio-degeneration produced small molecular terpenes and mono terpenes, increasing the total area of these minor molecules. The total of calculated (%) detected phenols increased from 90.08 to 92.92% after 10 kGy irradiation, as indicated in Fig (1). Because of their hydroxyl groups, phenols are particularly significant plant components because of their capacity to scavenge free radicals (Heim *et al.*, 2002 ; Alcalde *et al.*, 2019). Several investigations have discovered a substantial association between phenols and antioxidant activity (Silva *et al.*, 2006 ; Haung *et al.*, 2005)

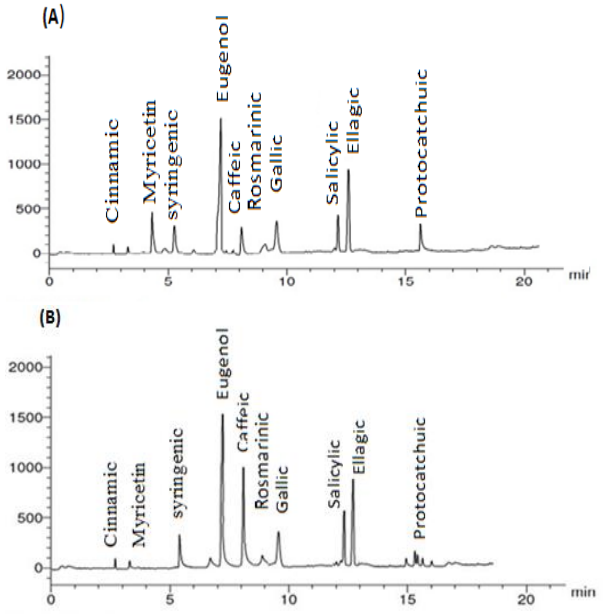

Fig. 1. HPLC chromatogram of individual phenolic compounds in solid residue extract of non-irradiated (A) and 10kGy irradiated clove buds (B).

Fractionation and identification of flavonoid compounds

The solid waste residues of un-irradiated and irradiated clove buds with 10 kGy and extracted with methanol (70%)and subjected to HPLC. Data in Fig. (2) showed that eight flavonoid compounds were identified. Chrysoeriol was the most abundant flavonoid compound, followed by hisperdin, quercitrin, catechin, luteolin, rutin, and naringin, with Kampferol being the least prevalent. γ-irradiation with 10 kGy increased the concentration of four flavonoids. For example, Rutin concentration increased from 5.23 to 8.14 mg/ml. Rutin has been linked to significantly lower hepatic and cardiac triglyceride levels (Fernandez *et al.*, 2010), and it is thought to have anti-inflammatory properties (Guardia *et al.*, 2001). Naringin increased from 4.12 to 7.04 mg/ml. Finally, quercetin increased from 9.21 to 10.04 mg/ml. Quercetin has been shown to have antioxidative, anti-aggregation, anti-inflammatory, and vasodilatory properties. (Erlund, 2004). Finally, the concentration of Kampferol increased from 4.05 to 5.17 mg/ml. According to Pereira *et al.* (2015), 10 kGy increased the extractability of flavonoid compounds in methanolic extracts of Ginkgo biloba samples and provided the highest level in quercetin and kampferol derivatives when compared to the control sample.

In contrast, gamma irradiation treatment with 10 KGy decreased four flavonoid compounds such luteolin , hisperdin, catechin and chrysoeriol were decreased from 6.09, 12.45, 7.14, and 22.08 to 5.88, 11.56, 6.52 and 20.75 mg/ml, respectively.

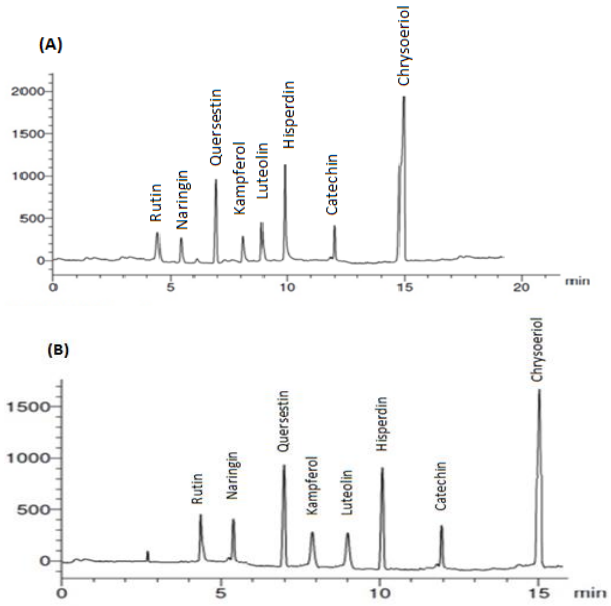

Fig. 2. HPLC chromatogram of individual flavonoid compounds in solid residue extract of non-irradiated (A) and 10kGy irradiated clove buds (B).

Oxidative stability of sunflower oil supplemented with methanolic extract of solid waste residue of irradiated clove buds at dose level of 10 kGy as a natural antioxidant:

Table (6) show the effect of clove solid residue on the oxidative stability of sunflower oil compared to the control sample (no antioxidant) and oil treated with BHT. All concentrations (100, 200, and 400 mg/L) of clove solid waste and BHT delayed the induction period (IP) of sunflower oil at 110ºC compared to the control sample, which was 6.77, 6.81, 6.95, 6.50, and 5.080 h, respectively.

Table 6. Oxidative stability of sunflower oil supplemented with various concentrations (100-400 mg/L) of methanolic extract of the solid waste residue of irradiated clove buds at a dose level of 10 kGy and BHT.

Treatments	Oxidative stability			
	Induction period at 110° C (hr)	Shelf life at 25°C (months)	Antioxidant activity (%)	Increasing index
Control	5.08	7.54	-	-
100 mg/L	6.77	10.04	1.33	33.26
200 mg/L	6.81	10.10	1.34	34.05
400 mg/L	6.95	10.31	1.37	36.81
BHT (200 mg/L)	6.5	9.62	1.28	27.95

Meanwhile, the oil sample with the lowest concentration (100 mg/L) displays greater oxidative stability than the sample containing BHT, increasing the computed parameters (shelf life at 25ºC and antioxidant activity), which were (10.04 and 9.62 months) and (1.33 and 1.28%), respectively. Otherwise, the sample containing 400 mg/L of clove solid waste demonstrated excellent oxidative stability metrics, boosting the antioxidant activity to 1.37% and extending the shelf life to 10.31 months.

The results are consistent with those of Lante *et al.* (2011), who examined soybean oil oxidative stability by contrasting BHT to lyophilized C.intybus var. 50% ethanol extract from silvestre leaves. The Rancimat test revealed that a small number of leaf extracts had greater antioxidant capacity than BHT, with antioxidant activity of 1.10% and an induction period of 9.12 hours at 110°C. Furthermore, Khalaf *et al.* (2018) compared sunflower oil that had been supplemented with BHT and control samples (antioxidant-free) to see if chicory leaf extract affected the oil's ability to resist oxidation. In terms of oxidative stability, the sample that contained both 1% and 2% leaf extract showed good results, increasing the antioxidant activity to 1.34% and extending the shelf life to 9.14 months.

CONCLUSIONS

The results demonstrated that the methanolic extracts from post-distillation waste (solid waste residue) of clove buds rich in phytochemicals and regarded as a valuable source of antioxidant agents. Gamma radiation dose of up to 10 kGy can boost the TPC and TFC of extracts, as well as their antioxidant activity. As a result, it would play a critical function in the fight of the antioxidant defense system against free radicals. Furthermore, these findings provide a solid foundation for the use of these plant wastes in future pharmaceutical and food industries.

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الانشطة المضادة للأكسدة ومحتوى الفينولات الكلية للمخلفات الصلبة الناتجة من التقطير المائى لنبات القرنفل المعامل بأشعة جاما.

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الملخص

أجريت هذه الدراسة بغرض تقييم النشاط المضاد للأكسدة للمخلفات الصلبة الناتجة بعد استخلاص الزيت الطيار لنبات القرنفل المعامل بأشعة جاما بجرعات صفر، 5 و 10 كيلو جراى . وتم تقدير المحتوى الكلى من الفينولات والفلافونيدات في المستخلص الميثانولى ومن ثم تفريد والتعرف على هذه المركبات بواسطة جهاز الكروماتوجرافيا السائلة عالية الكثافة (HPLC)كذلك تم تقدير النشاط المضاد للأكسدة بطريقتى القدرة الاختزالية للشقوق الحرة والقدرة الاختزالية لأيونات الحديديك . وقد أظهرت النتائج المتحصل عليها أن الجرعة الإشعاعية 10 كيلو جراى ادت الى زيادة معنوية فى المحتوى من المواد الفينولية والفلافونيدية وكذلك النشاط المضاد للأكسدة للمخلفات الصلبة لنبات القرنفل .كما اوضحت النتائج ان هناك عشرة مركبات فينولية وثمانية مركبات فلافونيدية وعلية فقد تم عمل عدة تركيزات (100، 200، 400 ملجم/ لتر) من الجرعة الإشعاعية 10 كيلو جراى واستخدامها كمادة مضافة طبيعية لدراسة تأثير إضافتها على الثبات التأكسدى لزيت زهرة دوار الشمس . وأوضحت النتائج أن إضافة التركيز 400 ملجم/ لتر أدى إلى زيادة الثبات التأكسدى لعينات الزيت (6.95 ساعة) . وبذلك يتضح من النتائج المتحصل عليها ان المخلفات الصلبة الناتجة بعد استخلاص الزيت الطيار لنبات القرنفل مصدر هام للمركبات ذات النشاط الحيوى كما تعتبر ايضا مصدرا للمواد الطبيعية المضادة للأكسدة.