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## In vitro screening of waste product of six plants for their antioxidant potential and cytotoxic effect on normal human skin fibroblast cell line (BJ1) and hepatocellular carcinoma cell line (HPG2)

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### Abstract

**Background:** Six cultivated and five wild families of plants that grow in Egypt have been randomly selected from various locations. The methanol plant extracts of *Polygonum aviculare*, *Rumex crispus*, *Ricinus communis*, *Ipomoea batatas*, *Ipomoea tricolor*, and *Achillea fragmentisma* were preliminary screened for their *in vitro* antioxidant and cytotoxicity activities. **Materials and Methods:** The extracts were assessed against hepatocellular carcinoma human cell line (HPG2). By reducing yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide to purple formazan in a mitochondrial manner, cell viability was evaluated. A known cytotoxic adrinamycin (doxorubicin) was used as a positive control. Additionally, the extracts were also determined against normal human skin fibroblast cell line (BJ1) using the same techniques. The antioxidant activity was evaluated using DPPH. **Results:** The present results of antitumor activity of different extracts declared 100.00, 70.60 and 55.20 % inhibition activity of extracts against HPG2 cells (at 100ppm) for *Ipomoea batatas*, *Ipomoea tricolor* and *Achillea fragmentisma* with IC<sub>50</sub> 28.5, 75.2 and 90.3 µg/ml, respectively as compared to standard drug which exhibit 100% reduction in HPG2 cells at 100 ppm with IC<sub>50</sub> 21.60 µg/ml. While, the extract of other investigated plants have showed low inhibitory activities against HPG2 cells with range 10.00- 15.00 %.

Further, the cytotoxic activity of different extracts on BJ1 recorded low cytotoxic activity for *Ipomoea batatas*, *Ipomoea tricolor* 15.00 and 10.00%, with  $IC_{50}$  31.4 and 49.60  $\mu\text{g/ml}$  respectively. *Achillea fragmentisma* showed more or less high cytotoxic activity 64.50% with  $IC_{50}$  43.4  $\mu\text{g/ml}$ . Also, *Polygonum aviculare*, *Ricinus communis* and *Rumex crispus* exhibited low cytotoxic activity against BJ1 (15.30, 18.20 and 14.50% with  $IC_{50}$  90.61, 70.10 and 95.00%, respectively). The results of antioxidant activity using DPPH assay revealed that both *Ipomoea* species have potential scavenging activity at different concentrations compared with other investigated plants. **Conclusion:** *Ipomoea batatas* and *I. tricolor* showed potent antitumor activity against HPG2 cells with low cytotoxic effects on BJ1.

**Keywords:** *Achillea fragmentisma*, *Ipomoea batatas*, *Ipomoea tricolor*, *Polygonum aviculare*, *Ricinus communis*, *Rumex crispus*, BJ1, HPG2, DPPH

الفحص المختبري المستخلصات نباتات طبيه ومعرفة قدرتها كمضادات أكسدة وتأثيرها السام  
علي الخلايا الليفية البشرية الطبيعية والخلايا الكبدية السرطانية

#### الملخص العربي

لقد وقع الاختيار لستة من النباتات التي تنمو في مصر بشكل عشوائي من مواقع مختلفة والتي تنتمي لخمس عائلات برية وتم عمل المستخلص الميثانولي لكلا من اوراق نبات الاشيليا واوراق نبات البطاطا الحلوة وأوراق البطاطا ثلاثية الألوان وأوراق نبات الخروع والحميض واوراق خاتم سليمان . لمعرفة انشطتها المضادة للأكسدة . وتأثيرها السام علي الخلايا الحية للكائن الحي والخلايا الكبدية السرطانية

**المواد والطرق المستخدمة:** تم تقييم المستخلصات ضد خطر الخلايا البشرية المصابة بسرطان الخلايا الكبدية وقابلية الخلايا للبقاء علي قيد الحياة ومقارنتها بالعلاج الكيماوي (الدوكسوروبيسن) كعنصر تحكم ايجابي. بالاضافة إلي ذلك تم تحديد المستخلصات ضد خط الخلايا الليفية الجلدية البشرية الطبيعية بإستخدام نفس التقنيات وكذلك تم تقييم النشاط المضاد للأكسدة لكل المستخلصات النباتية.

أظهرت النتائج الحالية : أن النشاط المضاد للأورام للمستخلصات النباتية المختلفة نشاط تثبيطي بنسبة ١٠٠% لمستخلص نبات الاشيليا مع تركيز دوائي ٢٨.٥ ميكروجرام/مل . وحقق مستخلص أوراق البطاطا ثلاثية الالوان نشاط تثبيطي بنسبة ٧٠.٦٠% مع تركيز دوائي بنسبة ٧٥.٢ ميكروجرام/مل. وكذلك حقق مستخلص اوراق نبات البطاطا الحلوة نشاط تثبيطي بنسبة ٥٥.٢٠% مع تركيز دوائي بنسبة ٩٠.٣ ميكروجرام/مل. مقارنة بالعقار القياسي الدوائي الذي

أظهر انخفاضاً بنسبة ١٠٠% للخلايا الكبدية السرطانية مع تركيز دوائي ٢١.٦٠% ميكروجرام/مل. في حين أظهرت المستخلصات النباتية الأخرى محل الدراسة نشاط تثبيطي منخفض ضد الخلايا الكبدية السرطانية بنسبة ١٠-١٥%. وعلي النحو الآخر بالنسبة للخلايا البشرية الطبيعية ومدى تأثير المستخلصات النباتية المختلفة عليها. فقد حقق المستخلص الميثانولي لأوراق البطاطا الحلوة سمية للخلايا الطبيعية بنسبة ١٥% مع تركيز دوائي بنسبة ٣١.٤ ميكروجرام/مل. وحقق مستخلص أوراق البطاطا ثلاثية اللون ١٠% مع تركيز دوائي بنسبة ٤٩.٦٠ ميكروجرام/مل. أظهر مستخلص نبات الاشيليا نشاطاً ساماً للخلايا مرتفع بنسبة ٦٤.٥% تركيز دوائي بنسبة ٤٣.٤ ميكروجرام/مل. كذلك أظهر مستخلص نبات الخروع والحامض وخاتم سليمان نشاطاً ساماً منخفض ضد الخلايا الطبيعية. كذلك كشفت نتائج الكشف عن مضادات الأكسدة للمستخلصات أن أعلى نسبة كانت لمستخلصات نبات أوراق البطاطا الحلوة والبطاطا ثلاثية اللون عند تركيزات مختلفة مقارنة بالمستخلصات الأخرى التي تم التحقق فيها.

**الخلاصة:** أظهر مستخلص أوراق البطاطا الحلوة والبطاطا ثلاثية اللون نشاطاً قوياً مضاداً للأورام ضد خلايا السرطانية الكبدية مع تأثيرات سامة منخفضة للخلايا الطبيعية.

## 1. Introduction

Herbal remedies are currently used by most people worldwide as part of their medical system, suggesting that plant-based traditional medicine will remain a significant part of human healthcare in the years to come (Meulmeester *et al.*, 2022). The main driving force behind the use of alternative therapies in search of a safer and more effective cancer cure may have been the increased adverse effects brought on by a number of cancer chemotherapeutic treatments.

As negative effects of chemically synthesized drugs developed, many authors resorted to ethno-pharmacognosy. They discovered that many plant phytochemicals were safe, had fewer negative effects, and were generally more effective than other options. Numerous positive biological effects have been documented, including anticancer, antimicrobial, antioxidant, antidiarrheal, analgesic, and wound-healing properties. People frequently assert that certain natural or herbal products have positive health effects. This sparked an ongoing hunt for novel substances with advantageous biological properties like antioxidant and anticancer (Souguir *et al.*, 2015). Compared to screening pure compounds isolated from the products, screening programs that select crude plant extracts may be more successful in their steps (Heckmann *et al.*, 2024).

Plants have long been known to have anticancer effects (Jiang *et al.*, 2022). Polyphenolics are secondary metabolites that exhibit a wide range of biological effects eliciting health benefit. Higher plants typically

respond to various biotic or abiotic stresses by stimulating their synthesis and accumulation (Ayeleso *et al.*, 2017; Jiang *et al.*, 2022). Flavonoids and polyphenolics have previously reported with potent anticancer activity with different regulation mechanisms including induction of apoptosis, free radical scavenging, and antiproliferative activities (Awad *et al.*, 2014; El-Baz *et al.*, 2017; Jiang *et al.*, 2022).

Phenolic compounds found in medicinal plants have been demonstrated to have positive health effects and to help prevent chronic illnesses (Jiang *et al.*, 2022). Egypt characterized by variable medicinal plants rich in polyphenolics and flavonoids. The current study demonstrated the evaluation of six selected plants *Polygonum aviculare*, *Rumex crispus*, *Ricinus communis*, *Ipomoea batatas*, *Ipomoea tricolor*, and *Achillea fragmentisma* for their antioxidant activities using 1, 1-diphenyl-2-picrylhydrazyl (DPPH), cytotoxic effects on hepatocellular carcinoma (HepG2) and normal skin fibroblast cell lines (BJ-1), using MTT assay.

## 2. Materials and Methods

### 2.1. Chemicals

All of the chemicals, including the standard ascorbic acid and 1,1-diphenyl-1-picrylhydrazyl (DPPH), were acquired from Fluka (Switzerland), Merck (Germany), BDH (England), and Sigma Chemical Company (St. Louis, MO, USA). Every chemical used was of the caliber of an analytical reagent. Ascorbic acid, dimethyl sulfoxide (DMSO), dichloromethane (DCM), methanol, 1-diphenyl-2-picrylhydrazyl (DPPH), 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide (MTT), and Eagle's Minimum Essential Medium (EMEM) were among the reagents that were purchased.

### 2.2. Apparatus

The antioxidant assay was conducted using a JASCO Corporation Model V-730 Spectrophotometer (S.N. A112961798, Tokyo, Japan). The extract was dried using a rotary vacuum evaporator (Laborota-4011, Heidolph Co., Germany) at 40 °C and low pressure. Aqueous alcohol was utilized to extract plant materials using a continuous extraction apparatus. To test the cytotoxic effect on human cell lines, a carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA) was utilized. The sterile space used to assess the cytotoxic effects on cell lines was a laminar flow cabinet biosafety class II level (Baker, SG403INT, Sanford, ME, USA).

### 2.3. Plant materials

A total of six plants were chosen. The plants under investigation, their families, and the parts used for the study were displayed in Table 1. In April, *Ipomoea batatas* Lam leaves and *Ipomoea tricolor* (Cav.) flowers are harvested from El Qanater El Khayreya in the El Qalyubiya Governorate, Egypt, while they are in the flowering stage. Mrs. Threase

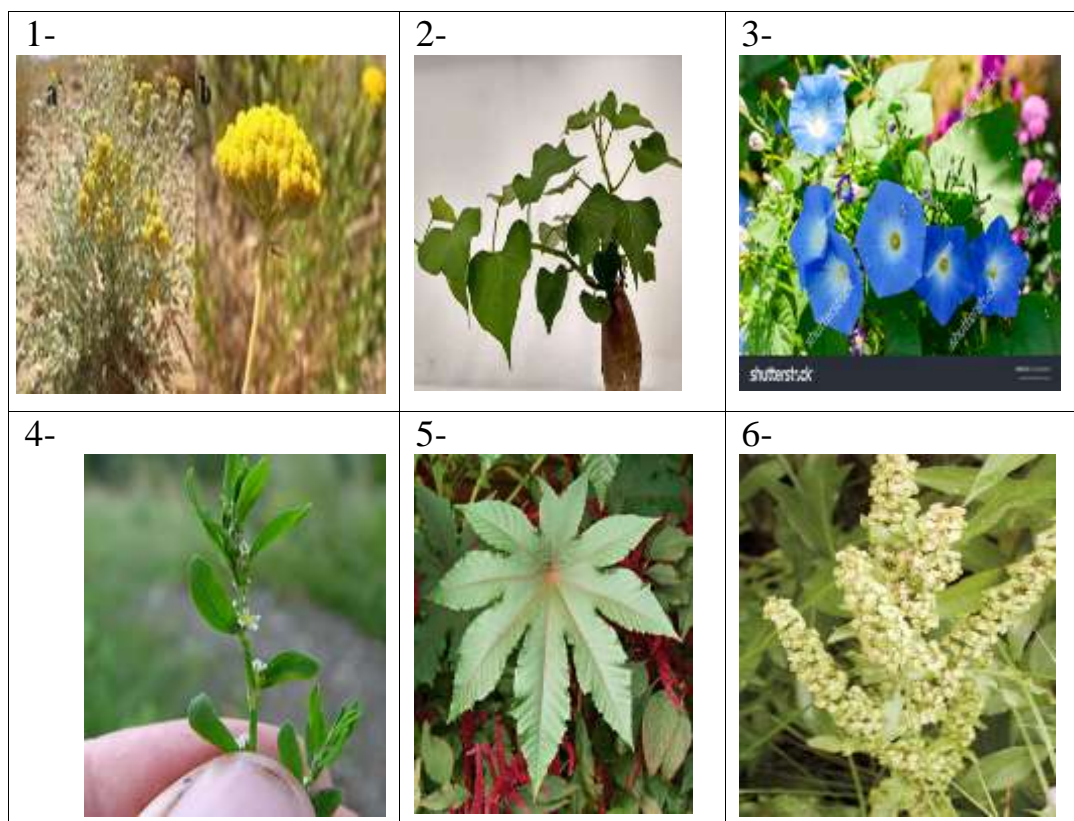


Labib, the Ministry of Agriculture's plant taxonomy consultant, identified the plant materials. The NRC's Department of Chemistry of Natural Compounds housed the voucher specimens. The collected leaves were stored in tightly sealed containers after being air dried and separately powdered. Photographs of the chosen medicinal plants were displayed in Figure 1. During the flowering season in April, *Achillea fragrantissima* Sch. Bip. (Family: Asteraceae) flowers were gathered from the Sinai desert on Wadi El-Gady, Egypt. According to **Boulos (2009)**, Dr. Mohammed El-Gibali verified the materials. Voucher specimens were placed in the National Research Centre's (CAIRC) herbarium in Dokki, Giza, Egypt. During the first flowering stage in April, the leaves of *Rumex crispus* and *Ricinus communis* L. (Family: Euphorbiaceae) were gathered from El-Gharbia Governorate (Berma, Tanta District), Egypt. *Polygonum aviculare* L. aerial parts (leaves and stems) were sampled from wet soil in Egypt's Mediterranean coastal region. Senior botanist El-Khanagry of the Horticultural Research Institute's Department of Flora and Phytotaxonomy Research verified the plants. Voucher specimens were placed in the National Research Center's herbarium in Giza, Egypt. Following air drying, powdering, and reduction to mesh no. 36, the plants gathered for the study were stored in tightly sealed containers.

**Table 1.** The investigated plants, their families and part used for the study

plants extract	Name of plant	Family	Part used
1	<i>Ipomoea batatas</i> L. Lam	Convolvulaceae	Leaves
2	<i>Polygonum aviculare</i> L.	Polygonaceae	Leaves and stems
3	<i>Ipomoea tricolor</i> (Cav.)	Convolvulaceae	Flowers
4	<i>Achillea fragmentisma</i>	Asteraceae	Flowers
5	<i>Ricinus communis</i> L.	Euphorbiaceae	Leaves
6	<i>Rumex crispus</i>	Polygonaceae	Leaves

Six plants were selected; *Achillea fragmentisma*, *Ipomoea batatas*, *Ipomoea tricolor*, *Polygonum aviculare*, *Ricinus communis*, and *Rumex crispus*. **Figure 1** showed photographs of the selected medicinal plants.



1: *Achillea fragmentisma* flowers; 2: *Ipomoea batatas* leaves; 3: *Ipomoea tricolor* flowers; 4: *Polygonum aviculare* and stems; 5: *Ricinus communis* leaves; 6: *Rumex crispus* leaves.

**Figure 1.** Photographs of the selected medicinal plants

#### 2.4. Preparation of aqueous alcoholic extracts

In a continuous extraction apparatus, a 50 g finely powdered, air-dried sample of each plant waste was extracted using 70% ethanol/water, and a rotatory evaporator was used to evaporate the mixture at a lower temperature and pressure (Fahmi *et al.*, 2019). Every extract was dried over anhydrous  $\text{CaCl}_2$  to a constant weight in a vacuum desiccator. The air-dried weight of the plant material was used to express the percentage yield of the sticky residue that ranged from dark brown to greenish brown. Each sample's yield of aqueous alcohol extract was reported.

#### 2.5. Effect of cytotoxicity on human cell lines of hepatocellular carcinoma (HPG2)

The mitochondrial-dependent conversion of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) from yellow to purple formazan was used to measure cell viability (Mosmann, 1983). Using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Sanford, ME, USA), all of the following procedures were carried out in a sterile environment. The cells were kept at 37 °C with 5%  $\text{CO}_2$  in Eagle's Minimum Essential Medium (EMEM) for HepG2, 1% L-glutamine, and an antibiotic-antimycotic mixture that contained 10,000U/ml potassium

penicillin, 10,000µg/ml streptomycin sulfate, and 25µg/ml amphotericin B. Using a water jacketed carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA), cells were batch cultured for 10 days before being seeded at a concentration of  $10 \times 10^3$  cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37 °C for 24 hours under 5% CO<sub>2</sub>. A final concentration of 100-50-25-12.5-6.25-3.125-0.78 and 1.56 µg/ml was obtained by aspirating the media, adding fresh medium (without serum), and then incubating the cells either by themselves (negative control) or with varying concentrations of the sample. The medium was aspirated after 48 hours of incubation, 40µl of MTT salt (2.5µg/ml) was added to each well, and the wells were then incubated for an additional four hours at 37°C with 5% CO<sub>2</sub>. Each well received 200µL of 10% sodium dodecyl sulphate (SDS) in deionized water, which was then incubated at 37°C for the entire night in order to halt the reaction and dissolve the crystals that had formed. As a known cytotoxic natural agent that provides 100% lethality under the same conditions, a positive control consisting of 100µg/ml was employed (Thabrew *et al.*, 1997; Mokbel *et al.*, 2024).

A microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) was then used to measure the absorbance at 595 nm with a reference wavelength of 620 nm. The SPSS 11 software's independent t-test was used to determine whether there was a statistically significant difference between the samples and the negative control (cells with vehicle). Plant extracts are dissolved in DMSO, which had a final concentration of less than 0.2% on the cells. The following formula was used to determine the percentage change in viability: ((reading of extract / reading of negative control) - 1) x 100.

Using the SPSS 11 software, a probit analysis was performed to determine the IC<sub>50</sub> and IC<sub>90</sub>. Control that is positive For the HEPG2 cell line, Adrinamycin (Doxorubicin) [Mw=579.99] has an IC<sub>50</sub> µg/ml of 21.6 and an IC<sub>50</sub> µM of 37.8.

## 2.6. Impact of cytotoxicity on normal human fibroblast cell line (BJ1)

The viability of cell was assessed by MTT (Mosmann, 1983; Galal and Mahmoud, 2021). The following procedures were done in same condition like the evaluation of the cytotoxic effect on hepatocellular carcinoma human cell lines (HPG2). Consideration was given to a sterile area with a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Sanford, ME, USA). At 37 oC with 5% CO<sub>2</sub>, cells were suspended in DMEM-F12 medium, 1% L-glutamine, and an antibiotic-antimycotic mixture containing 10,000U/ml potassium penicillin, 10,000µg/ml streptomycin sulfate, and 25µg/ml amphotericin B.



Using a water jacketed carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA), cells were batch cultured for 10 days before being seeded at a concentration of  $10 \times 10^3$  cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37 °C for 24 hours under 5% CO<sub>2</sub>. A final concentration of 100-50-25-12.5-6.25-3.125-0.78 and 1.56 µg/ml was obtained by aspirating the media, adding fresh medium (without serum), and then incubating the cells either by themselves (negative control) or with varying concentrations of the sample. The medium was aspirated after 48 hours of incubation, 40 µl of MTT salt (2.5 µg/ml) was added to each well, and the wells were then incubated for an additional four hours at 37°C with 5% CO<sub>2</sub>. Each well received 200 µL of 10% SDS in deionized water, which was then incubated at 37°C for the entire night in order to halt the reaction and dissolve the crystals that had formed. Under the same conditions, DOX, which was used as a positive control, is 100% lethal at 100 µg/mL (Thabrew *et al.*, 1997).

A microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) was then used to measure the absorbance at 595 nm with a reference wavelength of 620 nm.

## 2.7 Percentage of change of cell viability

The following formula was used to determine the percentage change in cell viability:  $((\text{reading of extract} / \text{reading of negative control}) - 1) \times 100$ . Using the SPSS 11 software, a probit analysis was performed to determine the IC<sub>50</sub> and IC<sub>90</sub>.

## 2.8. In vitro scavenging DPPH antioxidant activity

The antioxidants activity of the six investigated medicinal plants compared to ascorbic acid (standard) were measured using a modified method (Saad *et al.*, 2024; Valko *et al.*, 2007) and described in our previous work (Ahmed *et al.*, 2021). In summary, 100 milliliters of absolute methanol were used to prepare a 0.1 milliliter solution of 1, 1-diphenyl-2-picrylhydrazyl (DPPH). A Whatman No. 1 (Grade 589/2) filter paper was used to filter the methanol extract. One milliliter of this solution was mixed with one milliliter of each extract sample and three different concentrations of ascorbic acid (the reference drug) (0.01, 0.05, and 0.1 µg/ml). Methanol (95%) was used as the blank, and a solution containing DPPH (3 mL) in 100 µL of methanol (without antioxidant) is frequently used as the negative control. For half an hour, the tubes were left in total darkness. In order to compare the absorbance to ascorbic acid as a standard, it was spectrophotometrically measured at 517 nm using a JASCO Corporation Model V-730, S.N. A112961798, Tokyo, Japan.

The percentage of antioxidants in each extract (Valko *et al.*, 2007); was calculated using the formula : The inhibition percentage of DPPH• scavenging activity is equal to  $[(Ac - As)/Ac] \times 100$ .

As is the absorbance of each extract sample, and  $A_c$  is the absorbance of the DPPH• solution (without the tested extract, or control).

## 2.9. Statistical significance study

The SPSS 11 software's independent t-test was used to determine whether there was a statistically significant difference between the samples and the negative control (cells with vehicle). Plant extracts are dissolved in DMSO, which had a final concentration of less than 0.2% on the cells. The mean  $\pm$ SD% of three replicates was used to represent the statistical analysis of DPPH. Two-way ANOVA (SPSS software, version 8) was used, along with post hoc analysis, LSD (Least Significant Difference), and a co-state computer program that determines which different letters are significant at  $P \leq 0.05$ .

## 3. RESULTS

Six plants from five families that are both cultivated and wild in Egypt were chosen at random from various locations (Table 1). The methanol extracts of *Polygonum aviculare*, *Rumex crispus*, *Ricinus communis*, *Ipomoea batatas*, *Ipomoea tricolor*, and *Achillea fragmentisma* were preliminary screened for their activities. The six samples were evaluated for their cytotoxic effect against the HepG2 and BJ-1. The study was expanded to use the DPPH assay to assess the six plants' potential antioxidant activities.

### 3.1. Hepatocellular carcinoma cell line (HePG 2) inhibition activity of six plants

The present results of antitumor activity of different extracts declared 100.00, 70.60 and 55.20 % inhibition activity of extracts against HPG2 cells (at 100ppm) for *Ipomoea batatas*, *I. tricolor*, and *Achillea fragmentisma* with  $IC_{50}$  28.5, 75.2 and 90.3  $\mu$ g/ml, respectively as compared to standard drug which exhibit 100% reduction at 100 ppm with  $IC_{50}$  21.60  $\mu$ g/ml (Table 2). However,  $IC_{90}$  was 49.80, 118.30, and 139.20  $\mu$ g/ml for *Ipomoea batatas*, *Ipomoea tricolor* and *Achillea fragmentisma*, respectively compared with the value 38.2  $\mu$ g/ml of the standard drug Adrinamycin (doxorubicin). While, the extract of other investigated plants; *Polygonum aviculare*, *Ricinus communis* and *Rumex crispus* have showed low inhibitory activities against HPG2 cells with range 10.00-15.00 % with  $IC_{50}$  range was 95-99  $\mu$ g/ml compared with standard drug Adrinamycin (Doxorubicin) which showed  $IC_{50}$  21.6  $\mu$ g/ml. The three plants *P. aviculare*, *R. communis*, and *R. crispus* have showed  $IC_{90}$  of 129.70, 156.10, and 155.90  $\mu$ g/ml, respectively compared with the standard drug adrinamycin ( $IC_{90}$  37.8  $\mu$ g/ml).

**Table 2.** Inhibition activity of six plants against human hepatocellular carcinoma cell line (HePG 2)

plants extract	IC <sub>50</sub> (µg/mL)	IC <sub>90</sub> (µg/mL)	Inhibition percent % at 100 ppm
<i>Achillea fragmentisma</i>	90.30	139.20	55.20
<i>Ipomoea batatas</i>	28.50	49.80	100
<i>Ipomoea tricolor</i>	75.20	118.30	70.60
<i>Polygonum aviculare</i>	98.20	129.70	12.50
<i>Ricinus communis</i>	95.00	156.10	15.00
<i>Rumex crispus</i>	99.00	155.90	10.00
DMSO	-----	-----	1%
Positive control Adrinamycin (Doxorubicin)	21.6	38.2	100
Negative control	-----	-----	0 %

Using the MTT assay, sample concentrations range from 100 to 0.78 µg/ml; IC<sub>50</sub> is the lethal concentration of the sample that kills 50% of cells in 48 hours, and IC<sub>90</sub> is the lethal concentration of the sample that kills 90% of cells in 48 hours.

### 3.2. Cytotoxic activity test

#### 3.2.1. Potential cytotoxic activity of the six plants on normal human skin fibroblast cell line BJ1

The cytotoxic activity of different extracts on BJ1 recorded low cytotoxic activity for *I. batatas*, *I. tricolor* (15.20% and 10.00%), with IC<sub>50</sub> 31.40, 49.60 µg/ml and IC<sub>90</sub> 53.91, 71.90 µg/ml respectively (**Table 3**). *A. fragmentisma* showed high cytotoxic activity (64.50%) with IC<sub>50</sub> 43.40 µg/ml and IC<sub>90</sub> 66.12 µg/ml. Additionally, *P. aviculare*, *R. communis* and *R. crispus* exhibited low cytotoxic activity against BJ1 (15.30, 18.20 and 14.50% with IC<sub>50</sub> 90.61, 70.10 and 95.00%, respectively). While the values of IC<sub>90</sub> were 97.20, 88.97, and 109.82 µg/ml for *P. aviculare*, *R. communis*, and *R. crispus* respectively. *I. batatas*, *P. aviculare*, and *R. crispus* have showed nearly the same cytotoxic activity but with different IC<sub>50</sub> values (31.40, 90.61, and 95.00 µg/ml, respectively).

**Table 3.** Determination of cytotoxic activity of the six plants against the normal human skin fibroblast (1- BJ1)

plants extract	IC <sub>50</sub> (µg/ml)	IC <sub>90</sub> (µg/ml)	Cytotoxic activity(%)at 100ppm
<i>Achillea fragmentisma</i>	43.40	66.12	64.50
<i>Ipomoea batatas</i>	31.40	53.91	15.20
<i>Ipomoea tricolor</i>	49.60	71.90	10.00
<i>Polygonum aviculare</i>	90.61	97.20	15.40
<i>Ricinus communis</i>	70.10	88.97	18.20
<i>Rumex crispus</i>	95.00	109.82	14.50

DMSO	-----	-----	1%
Negative control	-----	-----	0 %

Using the MTT assay, sample concentrations range from 100 to 0.78 µg/ml; IC<sub>50</sub>: The sample's lethal concentration at which 50% of cells die within 48 hours; IC<sub>90</sub>: The sample's lethal concentration at which 90% of cells die within 48 hours.

### 3.2.2. Antioxidant activity

The potential scavenging of the free radical DPPH was used to gauge the antioxidant activity. (Table 4). The highest antioxidant activity was reported for *A. fragmentisma* (97.88±4.2%) followed by *I. batatas* (89.46±3.2%). The third extract order of scavenging activity was reported for *R. crispus* (79.32±1.98%), compared with standard vitamin C (ascorbic acid), which recorded DPPH scavenging activity of 90.79±1.3%. Regarding the percentage of DPPH inhibition of the three plants; *I. tricolor*, *P. aviculare* and *R. communis* was noticed to be more or less similar (50.87±2.5%, 47.89±0.2%, and 43.85±0.6%, respectively).

**Table 4.** Screening of DPPH scavenging activity of the six investigated medicinal plants extract

plants extract	Mean ±SD inhibition%
<i>Achillea fragmentisma</i>	97.88±4.20 <sup>c</sup>
<i>Ipomoea batatas</i>	89.46±3.2 <sup>b</sup>
<i>Ipomoea tricolor</i>	50.87±2.50 <sup>a</sup>
<i>Polygonum aviculare</i>	47.89±0.20 <sup>a</sup>
<i>Ricinus communis</i>	43.85±0.60 <sup>a</sup>
<i>Rumex crispus</i>	79.32±1.98 <sup>d</sup>
Ascorbic acid	90.79±1.30 <sup>b</sup>

The mean ±SD of three replicates is used to represent the data. Two-way ANOVA (SPSS software, version 8) and post hoc Least Significant Difference (LSD) are used in statistical analysis, along with the Co-state software, which determines which different letters are significant at  $P \leq 0.05$ .

## 4. Discussion

The use of natural products derived from plants to slow the progression of cancer has recently garnered a lot of attention from researchers. All the samples were assessed for their cytotoxic effects on liver carcinoma and normal skin fibroblast cell lines using MTT assay.

Respecting to hepatocellular carcinoma, the extracts of *I. batatas*, *I. tricolor* and *A. fragmentisma*, respectively have markedly potent cytotoxic activities on HPG2 cell lines compared with the standard Adrinamycin (Doxorubicin) with IC<sub>50</sub> = 28.5, 75.2 and 90.3 µg/ml versus 21.60 µg/ml for Doxorubicin. While, IC<sub>90</sub> values were 49.80, 118.30, and 139.20 µg/ml respectively relative to IC<sub>90</sub> = 38.2 µg/ml of Doxorubicin (Table 2). The other three plants; *P. aviculare*, *R. communis* and *R. crispus* displayed low

cytotoxic activity against HePG2 with  $IC_{50}$  =99.00, 98.20 and 95.00  $\mu$ g/ml, respectively relative to the reference drug ( $IC_{50}$  =21.6  $\mu$ g/ml). While ,  $IC_{90}$  for the three extracts showed 129.70, 156.10, and 155.90  $\mu$ g/ml, respectively compared with the standard drug Adrinamycin (  $IC_{90}$  37.8  $\mu$ g/ml).

In the current study, the results of cytotoxic activity of the two *Ipomoea* extracts on BJ1 reported low cytotoxic activity for *I. batatas* and *I. tricolor* (15.20 and 10.00%, respectively), with  $IC_{50}$  31.4 and 49.60 $\mu$ g/ml respectively (**Table 3**). *I. tricolor* (Heavenly blue) is known in Arabic as Magd-Asabah and Leblaba. It was found to conatains resin glycosides, anthocyanins, alkaloids and resin glycosides Tricolorins are structurally amazing resin glycosides isolated from the plant with promising bioactivities. The plant is a weed controller and it was used as a cover crop in Mexico (**Pereda-Miranda et al, 1993**).

Our current study's findings support this plant's antitumor properties in vitro. A study by **Bağcı Uzun et al. (2022)** reported the anti-tumor effect of yarrow (*A.millefolium*) extract on the BALB/c mice in which Ehrlich solid tumor is induced (**Bağcı Uzun et al., 2022**). The extract was found to have a curative effect on necrosis, bleeding, and areas of inflammation in extract treatment groups alone. The extract has not showed any significant effect on the characters of the tumor (length, volume, growth, or its weight). In an in vitro study, **Babazade et al. (2022)** reported the synergistic anticancer effect on human pancreatic cancer cell line of metformin and *A. vermicularis* Trin-loaded nanofibers. **Abdalla et al. (2020)** recorded that *A. membranacea* has proapoptotic activity against A2780 ovarian cancer cells. **Machado and co-workers (2022)** have reported the monoterpenes such as 1,8-cineole, the major constituent from oil. The present results of antitumor activity of extract of *A.fragmentisma* is in agreement with a study that has shown pronounced cell proliferative effect of different fractions of leaves part of *A. fragrantissima* against T-cell lymphoma (Jurkat), human chronic myelogenous leukemia (CML) and hepatocellular carcinoma cell lines by MTT assay (**Patocka and Navratilova, 2019**). The activity of extract may be attributed to its phytoconstituents that have been shown to possess antioxidant activity (**Al-Mustafa and Al-Thunibat, 2008; Abd-Alla et al., 2021; Vitalini et al., 2022**). Yarrow (*A. millefolium*) L., the fragrant perennial herb has been used traditionally for its anti-inflammatory and wound-healing properties, was rich in bioactive flavonoids and sesquiterpenoid classes of guaianolide type. Achillinin a cytotoxic guaianolide from the plant's flowers has reported (**Li et al., 2011**). In another work, the ethyl acetate fraction of the same species of *Achillea* causes human cervical cancer (HeLa) cells to undergo apoptosis and cell cycle arrest (**Abou Baker et**



*al.*, 2020). At varying concentrations, the plant shoots (yarrow) and bleomycin had a synergistic effect on "normal" skin cells (HFFF2) and prostate cancer cells (DU145) (Shahani *et al.*, 2015). At doses of 2000 and 1000 µg/mL, respectively, a methanol extract from yarrow demonstrated a significantly higher cytotoxicity induced by bleomycin, with survival rates of 49 and 60% and was not toxic toward "normal" cells. This may indicate that this extract contains bioactive components able to minimize negative side-effects caused by toxicity toward "normal" cells, while improve the effectiveness of bleomycin. The contents of various plant extracts may be linked to their cytotoxic activity of cytotoxic molecules (Sweelam *et al.*, 2018; Abd-Alla *et al.*, 2021). *Achillea* species contain different classes of bioactive compounds, including flavonoids and terpenoids (Abd-Alla *et al.*, 2016). The presence of many cytotoxic molecules in yarrow *Achillea* species are reported such as the flavonoids casticin and apigenin as well as the phenolic acids, such as *p*-coumaric, chlorogenic, and rosmarinic acid. Our current findings supported *Achillea* extract's antitumor and antioxidant properties. Four methoxy flavones were isolated from *A. biebersteinii* (collected from Saudi Arabia) as a result of our earlier phytochemical analysis. The antioxidant effect of methoxy flavones has been explained by a number of activities (Awad *et al.* 2014). They have also been discovered to be scavengers of free radicals. The antitumor and antioxidant activities are well correlated with the polyphenolics and terpenes (Abd-Alla *et al.* 2016; Custodio *et al.*, 2022).

The present study revealed also, both *A. fragmentisma* and *I. batatas* exhibited potent antioxidant capacity as they exhibited noticeable free-radical scavenging activity ( $97.88 \pm 4.2\%$  and  $89.46 \pm 3.2\%$ , respectively) (Table 4). Family *A. fragmentisma* in Arabian traditional medicine, the Asteraceae family is traditionally used internally to treat wound healing, inflammatory and spasmodic gastrointestinal disorders, skin inflammations, and appetite-stimulating herbs.

(Ezzat *et al.*, 2014; Abd EL-Fattah *et al.*, 2018; Bashir *et al.*, 2022) as well as hypoglycemic (Cirak *et al.*, 2022). Numerous beneficial bioactive compounds that have been shown to have anti-inflammatory and antioxidant effects define this plant. Polyphenols, flavonoids, terpenes, and alkaloids were found to be the primary pharmacologically active components (Patocka and Navratilova, 2019).

*Achillea fragrantissima* is one of the important desert plants. It has been demonstrated that the phytochemicals found in desert plants can help prevent and treat complex diseases like cancer as well as unfavorable pathophysiological conditions. The constituents of *Achillea* are mainly terpenes, flavonoids, phenolic acids, sesquiterpene lactones, ionone

glucosides and terpenoids (Saeidnia *et al.*, 2011; Abd-Alla *et al.*, 2016; Cirak *et al.*, 2022).

Numerous pharmacological and phytochemical investigations have been conducted to demonstrate the significance of *A. fragrantissima*. *A. fragrantissima* was found to have the most significant  $\alpha$ -glucosidase inhibitory activity, lower postprandial glucose, and delay the absorption of ingested carbohydrates (Saeidnia *et al.*, 2011). Acacetin-6-C-(6''-acetyl- $\beta$ -Dglucopyranoside)-8-C- $\alpha$ -L-arabinopyranoside, cirsimaritin, chrysoplenol, cirsiol, eupatilin-7-methyl ether, and isovitexin 4'-methyl ether were among the flavonoids that were isolated (Ahmed *et al.*, 1988, 1989; Ezzat and Salama, 2014). Along with the bitter compound keissoside, the fatty acids lauric, myristic, palmitic, stearic, linoleic, linolenic, oleic, and diacyl glycerol of palmitic acid were also separated. *A. fragrantissima* contains a significant class of sesquiterpene lactones, which include 13-O-desacetyl-1- $\beta$ -hydroxyafraglouclide, achilloide A, and 1 $\alpha$ , 4 $\alpha$ -endoperoxypseudoguaia-7, 10-diene-6 $\beta$ , 12-olide. Additionally, acetates of taraxasterol and pseudotaraxasterol were found (Elgamal *et al.*, 1991; Moustafa *et al.*, 1995). Furthermore, 6-oxo-S-hydroxysantolina-1, 4-diene, 4, 5, 8-trihydroxy-santolin-1-ene, and 5, 8-epoxy-4, 6-dihydroxy-santofin-1-ene were detected in *A. fragrantissima* (Ahmed *et al.*, 1990).  $\alpha$  and  $\beta$ -thujone,  $\alpha$ -pinene,  $\beta$ -pinene, limonene, 1,8-cineole, linalool, carvacrol, eugenol, artemisia ketone, palustrol, sabinene hydrate,  $\alpha$ -terpineol, and santolina alcohol are the main constituents of the herb *A. fragrantissima*, which also contains a 0.81% essential oil. Its tannin content, which includes pyrocatechol, resorcin, phloroglucin, and methyl phloroglucin, reaches 8%. Additionally, flavonoids including afroside, cirsimartin, chrysoplenol, and cirsiol were reported (Bashir *et al.*, 2022).

In our present work, the flavones derivatives of luteolin and apigenin derivatives were isolated and identified from *A. fragrantissima*. Numerous reports showed that these class of compounds (flavones) exhibited different properties, such as anti-oxidative and antitumor (Xue *et al.*, 2022). These phytochemicals detoxify reactive oxygen species (ROS), which may prevent oxidative damage.

The plant *A. fragrantissima* is characterized by other bioactive constituents such as tannin content and has been reported reaches 8% (such as phloroglucin, pyrocatechol, resorcin, and methyl phloroglucin). This high content of tannins may act as antioxidants. They may help the endogenous antioxidant enzymes that are involved in the redox metal ions or scavenging inactivation of ROS prior to lipid peroxidation by reducing lipid peroxidation.

The sesquiterpenoids of type guaianolide and germacranolide (from *Achillea alpina*) were reported to reduce insulin resistance in palmitic

acid-treated HepG2 cells (Xue *et al.*, 2022). Alshuail *et al.* (2022) observed anti-proliferative and pro-apoptotic characteristics in MDA-MB-231, human triple-negative breast cancer cells of flowers methylene chloride extract of *A. fragrantissima*, which is consistent with our findings. It has been reported that an aqueous extract of *Achillea* species has a moderate antiproliferative effect on cultured melanoma cell lines (Sathiyamoorthy *et al.*, 2019). In this study, we found that an aqueous alcoholic extract from the *A. fragrantissima* plant has a strong cytotoxic effect. In another study, human cancer cell lines were used to test the toxicity of successive extracts of *Achillea*'s aerial parts using the MTT assay. These extracts' cytotoxic activity was proposed to be related to their polyphenolic content, which demonstrated the ability to block carcinogenic pathways by activating caspases (Anantharaju *et al.*, 2016; Abd-Alla *et al.*, 2021).

Further, the antioxidant capacity has been reported for the infusion prepared from fifteen *Achillea* species with protection against H<sub>2</sub>O<sub>2</sub>-induced oxidative damage in human erythrocytes and leukocytes (Aljaja *et al.*, 2021). The other interest plant with antioxidant activity was *I. batatas* (Sweet potato). *Ipomoea* waste materials contain proteins, carbohydrates, pigments, micro- and macronutrients, and phenolic compounds (Awad *et al.*, 2018). Morning glory family (Convolvulaceae) includes the important food plant sweet potatoes (*I. batatas* L.), which is a significant food crop in the world's subtropical areas. *I. batatas* and *I. tricolor* (stir fry mixes) can be eaten raw or cooked, in salads, stews, and soups. In Maori people the incidence of non-infectious diseases appears to have been low, perhaps in part because they eat sweet potato (or kumara) which has anti-cancer properties (Asadi, 2017). The genus *Ipomoea* (Convolvulaceae) thrives in tropical and subtropical regions of Asia, Africa, and America. It has been discovered that a number of extracts made up of different polyphenols can scavenge DPPH free radicals (Abd-Alla *et al.*, 2020). In the current work, we use ethanol (70%) as extracting solvent to prepare crude total extract of each plant. The recovery and antioxidant properties of the polyphenol components are significantly impacted by the type of extracting solvent used. Aqueous alcoholic (70%) extraction was shown in a prior study to be an effective solvent system for recovering polyphenols from *I. batatas* leaves (Islam *et al.*, 2024). It was discovered to be rich in nutritive and functional components, but it leaves part of the sweet potato, which is not typically used. The leaves have a high amino acid score and are high in protein. When compared to other vegetables, sweet potatoes' leaves were higher in soluble dietary fiber, vitamins B2, C, and E, and minerals, especially iron (Sun *et al.*, 2014, 2019; Jiang *et al.*, 2022). Different plant's parts of sweet potato gain

different phenolic profiles which have drawn researchers' attention for its unique health benefits **Jiang et al., 2022**). The leaves in particular is a great source of raw materials for phenolic compounds, which have strong antioxidant properties and can be used as natural antioxidants, food preservatives, or nutritional supplements added to food products. Carbohydrates, proteins, phenolic compounds, macro and micronutrients, and pigments are also present in sweet potato waste materials. Currently, high percentage of sweet potato leaves in Egypt (95–98%) is discarded as agricultural waste with low value. The remaining percentage (2–5%) is mainly used for excellent source of livestock feed (**Adeyeye et al., 2021**), which causes environmental pollution issues and massive resource waste. In this regard, recovering these inexpensive and widely accessible phenolic sources from sweet potato leaves may enhance their added value while also resolving the environmental issue these residues cause. A variety of health-promoting biological activities, such as antioxidative, anticancer, and anti-inflammatory properties, were demonstrated by the cellular and in vivo pharmacological evaluation of *I. batatas* (sweet potato) extract (**Zhang et al., 2020**). Sweet potato leaves are thus nutritional and functional foods (**Maghraby et al., 2005; Zhang et al., 2020**). Previous study showed that the total phenolic content (TPC) and total flavonoid content (TFC) in leaf of *I. batatas* ( $112.98 \pm 4.14$  mg gallic acid equivalent (GAE)/g of dried extract,  $56.87 \pm 5.69$  mg rutin equivalent (RE)/g of dried extract) (**Zhang et al., 2020**). We have been previously reported the potential *in vivo* immunomodulatory effect of *I. batatas* and *I. tricolor* (**Maghraby et al., 2005**). *I. batatas* are a valuable source of functional components as they are rich in proteins, minerals, vitamins, and other important components such as the polyphenols (**Maghraby et al., 2005; Zhang et al., 2020**). Of polyphenols, the biofunctional of flavonoids (e.g. apigenin, luteolin, myricetin, and quercetin, etc., the derivatives of caffeoylquinic acid (CQA) (including the 3, 4, 5-triCQA, di-CQA and the mono-CQA), and anthocyanins are the predominant components (**Sun et al., 2014, 2019; Zhang et al., 2020**). These polyphenols, particularly di-CQA and 3,4,5-triCQA, have potent antioxidant properties that include scavenging free radicals, inhibiting lipid peroxidation, and metal chelation (**Jiang et al., 2022**). The antioxidant activity of sweet potato leaves (Jishu No. 18) has also been studied among many other edible plants. Its reducing power (ferric ion reducing antioxidant power, or FRAP) was nearly 9.43, 6.14, 3.37, and times higher than that of common vegetables like broccoli, spinach, and green cabbage, respectively

(**Sun et al., 2019; Jiang et al., 2022**). The biological activities of Ipomoea species' extracted compounds, fractions, and extracts have been assessed



in a number of studies. Antinociceptive, anti-inflammatory, antiproliferative, antioxidant, antimicrobial, antispasmodic, collagenase inhibitory, anticancer, and multidrug-resistance efflux inhibitory activities are among the species' principal activities that have been documented (Akinniyi *et al.*, 2022; Jiang *et al.*, 2022). It was reported that *I. batatas* has a hepato-protective, antioxidative, antidiabetic (Ludvik *et al.*, 2002; Kusano and Abe, 2000; Matsui *et al.*, 2002) antimutagenicity (Yoshimoto *et al.*, 1999; Konczak-Islam *et al.*, 2003) and potential chemopreventive activities (Ishida *et al.* 2000). The glycolipids isolated from the plant leaves and stem was found to be cancer cell-proliferation-inhibiting or differentiation-promoting (Eicken *et al.*, 1998) and modify colorectal carcinogenesis in male F344/Du Crj rats (Yoshimoto *et al.*, 2002). In the current study, the percentage of DPPH inhibition of *I. tricolor*, *P. aviculare* and *R. communis* was nearly similar in inhibition activity with values  $50.87 \pm 2.50\%$ ,  $47.89 \pm 0.20\%$ , and  $43.85 \pm 0.6\%$ , respectively. *P. aviculare* has showed  $IC_{90}$  of 129.70  $\mu\text{g/ml}$  compared with the standard Adrinamycin ( $IC_{90}$  37.8  $\mu\text{g/ml}$ ) (Table 4). *P. aviculare* (subfamily Polygonoideae) is employed to treat some types of cancer in the folk medicine of Austria and China. The plant mainly used as an appetite stimulant in some countries (Costea and Tardif, 2005; Chon *et al.*, 2008). Common knotweed, or *P. aviculare*, is a plant that is used in Korean salads and is edible. In the Mediterranean coastal region, the plant could be harvested from moist soil. *P. aviculare*'s chemical characteristics have been extensively studied in recent decades, and data collected has demonstrated that its extracts are rich in sesquiterpenoids, flavonoids, tannins, and a variety of polyphenols, which has helped to establish its recognition in modern pharmacology and support its ethnopharmacological use (Thu *et al.*, 2004; Costea and Tardif, 2005; Chon *et al.*, 2008). The phytochemical studies' findings showed that tannins, saponins, flavonoids, alkaloids, and sesquiterpenes were present. In the present study, the three plants *P. aviculare*, *R. communis*, and *R. crispus* have showed  $IC_{90}$  of 129.70, 156.10, and 155.90  $\mu\text{g/ml}$ , respectively compared with the standard Adrinamycin ( $IC_{90}$  37.8  $\mu\text{g/ml}$ ). *R. crispus* (family Polygonaceae), the perennial flowering plant known as the curly dock, curled dock, or yellow dock is indigenous to Western Asia and Europe. The castor bean, also known as the castor oil plant, is a species of perennial flowering plant that belongs to the Euphorbiaceae spurge family. Excessive levels of free radicals in the human body have been linked to the emergence of a number of diseases (Ahmed *et al.*, 2011; Abd-Alla *et al.*, 2021). The important role of secondary metabolites to counteracting the effects of free radicals in the body, is well-known (Sweelam *et al.*, 2018; Abd-Alla *et al.*, 2020). Natural products have been



identified as a source of many antioxidant substances in past years, including the two investigated species *Ipomoea batatas* and *Ipomoea tricolor* (Jiang *et al.*, 2022). Our previous work (Abd-Alla, 2004; El-Hawary *et al.*, 2021) has demonstrated the identification of pentacyclic lupane-type (lupeol) and the pentacyclic ( $\beta$ -amyirin) triterpenes from the two *Ipomoea* species (*I. batatas* and *I. tricolor*). Numerous biological activities have been demonstrated for these triterpenes, including their protective effects against cancer, inflammation, and arthritis (Sweelam *et al.*, 2018; Abd-Alla *et al.*, 2020). Several pharmacologically active components, such as flavonoids, glycosides, terpenoids, steroids, and alkaloids have been previously revealed with phytochemical analyses of the plant (Akinniyi *et al.*, 2022). It was proposed that these phytoconstituents were in charge of the diverse range of biological activities that plant extracts possessed. Additionally, the methanol extract of aerial parts of different *Ipomoea* species yielded the triterpene  $\beta$ -amyirin and the phytosterol  $\beta$ -sitosterol (Akinniyi *et al.*, 2022). The same triterpene was extracted from *Symplocos cochinchinensis* leaves, where it showed impressive antioxidant properties (Sunil *et al.*, 2014). The study compare IC<sub>50</sub> value of ascorbic acid (0.437  $\mu$ M) and BHT (0.351  $\mu$ M) with the IC<sub>50</sub> value of  $\beta$ -amyirin (0.190  $\mu$ M) on superoxide antioxidant activity (Sunil *et al.*, 2014). However, Polygonum hydropiper's phytosterol also demonstrated potent free radical scavenging properties. The IC<sub>50</sub> values for the radical scavenging properties of  $\beta$ -sitosterol against hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), and DPPH were determined to be 0.675, 0.289, and 0.338  $\mu$ M, respectively. In contrast, the IC<sub>50</sub> values for the standard (ascorbic acid) are 0.369, 0.114, and 0.284  $\mu$ M, respectively (Akinniyi *et al.*, 2022).

From the acetone extract of *I. pes-caprae* leaves, a coumarin known as 5,7-dihydroxy-4-phenyl-2H-chromen-2-one was extracted, and it demonstrated notable and dose-dependent activity against hydroxyl radicals (IC<sub>50</sub> = 0.055  $\mu$ M) and DPPH radicals (IC<sub>50</sub> = 0.032  $\mu$ M) (Alagesan *et al.*, 2019). The coumarins have significant therapeutic potential, which is attributable to the free-OH groups at C8, C7, or C6 positions (Shalaby *et al.*, 2014; Alagesan *et al.*, 2019).

Both 4-*O*-caffeoylquinic acid and 3-*O*-caffeoylquinic acid, identified from a methanol fraction of aerial parts of *I. pes-caprae*, have showed radical scavenging properties (Ekeuku *et al.*, 2021; Akinniyi *et al.*, 2022). For instance, other isolated compound isoquercetin exhibited high antioxidant activity in a DPPH assay with value 0.048  $\mu$ M as a half-maximal radical scavenging level. Previous studies on quercetin 3-*O*-galactoside and caffeic acid also indicated their respective antioxidant properties (Ekeuku *et al.*, 2021). As

an antioxidant, caffeic acid ameliorates reactive oxygen species (**Abd-Alla et al., 2009**) Likewise, chlorogenic acid ( $IC_{50}$  of  $3.29\mu M$ ), isochlorogenic acid B ( $IC_{50}$  of  $3.79\mu M$ ), and isochlorogenic acid C ( $IC_{50}$  of  $10.45\mu M$ ), According to **Phull et al. (2016)**, which were purportedly isolated from *Bidens pilosa*, demonstrated the capacity to scavenge DPPH using quercetin and caffeic acid as reference compounds With an  $IC_{50}$  of  $0.033\mu M$ , in another study caffeic acid demonstrated a higher capacity to scavenge DPPH radicals than ascorbic acid ( $IC_{50} = 0.245\mu M$ ).

*I. pes-caprae* was the source of salicylic acid, another antioxidant compound. The compound functions as an inhibitor of oxidative stress by binding iron ( $Fe^{2+}$ ), which during lipid peroxidation generates peroxy, alkoxy, and OH- radicals. The fresh and dried leaves of *I. pes-caprae* are characterized by the presence of  $\alpha$ -terpineol, limonene, 8-cedren-13-ol,  $\beta$ -caryophyllene, and  $\alpha$ -copaene as some of the major constituents of their essential oil. The ferric-reducing antioxidant power (FRAP) and DPPH scavenging test, which were used to assess the terpenoid  $\beta$ -caryophyllene' capacity to scavenge radicals, yielded  $IC_{50}$  values of  $3.23$  and  $1.25\mu M$ , respectively, in contrast to ascorbic acid's  $3.8$  and  $1.5\mu M$ , respectively. These observations were in parallel with the results of *Citrus medica* for its antioxidant activities. The essential oil of *Peucedanum longifolium* is abundant in 8-cedren-13-ol, where it inhibited lipid peroxidation and exhibited a potent DPPH free-radical scavenging ability.

The highest antioxidant activity reported for *A. fragmentisma* in our study ( $97.88\pm 4.2\%$ ), was followed by *I. batatas* ( $89.46\pm 3.2\%$ ) as compared to ascorbic acid standard drug ( $90.79\pm 1.3\%$ ). Previous studies confirmed the contribution of plant-derived substances (such as phenols, flavonoids and tannins) with the displayed antioxidant activities by different mechanisms (**Awad et al., 2014**). However, the results indicated that *A. fragmentisma* ( $97.88\pm 4.2$ ) followed by *I. batatas* ( $89.46\pm 3.2$ ) enhance the potential antioxidant activity due to their high content of total phenolics and total flavonoids content. In this sense, the flavonoid content of *A. fragmentisma* and *I. batatas* correlates with their antioxidant activity. The high correlation coefficient value between its content and this plant extract's antioxidant activity served as confirmation of this.

**Table 6.** DPPH scavenging activity of some compounds identified in *Ipomoea batatas* and *Ipomoea tricolor*

Compound number	Name of compound	Class of compound	DPPH Scavenging value	Reference
1	$\beta$ -Amyrin	Terpenoid	$IC_{50} = 1.25\mu M$	Sunil et al., 2014; Akinniyi et al., 2022
2	$\beta$ -Caryophyllene	Terpenoid	$IC_{50} = 0.338\mu M$	Dahham et al., 2015

3	Quercetin	Flavonoid	IC <sub>50</sub> = 0.028 µM	Majewska et al., 2011
4	Quercetin 3-O-glucoside (isoquercetin)	Flavonoid	RC <sub>50</sub> = 0.048 µM	Gao et al., 2019; Qasim et al., 2017
5	5,7-Dihydroxy-4-phenyl-2H-chromen-2-one	Coumarin (Chromenone)	IC <sub>50</sub> = 0.032 µM	Alagesan et al., 2019; Akinniyi et al., 2022
6	isochlorogenic acid B (3,4-di-O-caffeoylquinic acid)	Phenolic acid	(IC <sub>50</sub> = 3.29 µM)	Qasim et al., 2017
7	Caffeic acid	Phenolic acid	IC <sub>50</sub> = 0.033 µM	Qasim et al., 2017
8	β-sitosterol	Phytosterol	IC <sub>50</sub> = 0.338 µM	Akinniyi et al., 2022
8	α-Terpineol	Terpenoid	IC <sub>50</sub> = 1.03 µM	Sunil et al., 2014; Akinniyi et al., 2022
9	α-Copaene	Terpenoid	IC <sub>50</sub> = 1.42 µM	Sunil et al., 2014; Akinniyi et al., 2022
10	8-Cedren-13-ol	Sesquiterpene alcohol	IC <sub>50</sub> = 1.05 µM	Sunil et al., 2014; Akinniyi et al., 2022
11	Isochlorogenic acid C (4,5-di-O-caffeoylquinic acid)	Phenolic acid	IC <sub>50</sub> = 3.79 µM	Qasim et al., 2017
12	Chlorogenic acid (5-O-caffeoylquinic acid)	Phenolic acid	IC <sub>50</sub> = 10.45 µM	Qasim et al., 2017
<b>Standard antioxidants</b>	Vitamin C/ Ascorbic acid	Standard antioxidant	IC <sub>50</sub> = 0.111 µM/ 0.245 µM	Qasim et al., 2017; Akinniyi et al., 2022
	1. Trolox	Standard antioxidant	IC <sub>50</sub> = 0.15 µM	Akinniyi et al., 2022
	2. Butylated hydroxyanisole (BHA)	Synthetic antioxidant	IC <sub>50</sub> = 42.15 µg/mL	Sunil et al., 2014; Akinniyi et al., 2022
	3. Butylated hydroxytoluene (BHT)	Synthetic antioxidant	IC <sub>50</sub> = 35.24 µg/mL	Akinniyi et al., 2022

DPPH: 2,2-diphenyl-1-picrylhydrazyl; RC<sub>50</sub>: half-maximal radical scavenging concentration; IC<sub>50</sub>: half-maximal inhibitory concentration

## Conclusion

Six wild and cultivated plants from five families that are found in Egypt were chosen at random from various locations.. The methanol plant extracts of *P. aviculare*, *R. crispus*, *R. communis*, *I. batatas*, *I. tricolor*, and *A. fragmentisma* were preliminary screened for their activities. The six samples were evaluated for their cytotoxic effect against the HepG2 and BJ-1. The study was extended to evaluate the potential of antioxidant

activities of the six plants using DPPH assay. The present results of antitumor activity of different extracts declared the highest inhibition activity of extracts against HPG2 cells for *I. batatas*, *I. tricolor*, and *A. fragmentisma* respectively as compared to standard drug (Doxorubicin). While, the extract of other investigated plants; *P. aviculare*, *R. communis* and *R. crispus* showed low inhibitory activities. The cytotoxic activity of different extracts on BJ1 recorded low cytotoxic activity for *I. batatas*, *I. tricolor* respectively. Although, *A. fragmentisma* showed high cytotoxic activity. Additionally, *P. aviculare*, *R. communis* and *R. crispus* exhibited low cytotoxic activity against BJ1. *I. batatas*, *P. aviculare*, and *R. crispus* have showed nearly the same cytotoxic activity. The highest antioxidant activity was reported for *A. fragmentisma* followed by *I. batatas*. The third extract order of scavenging activity was reported for *R. crispus* compared with standard vitamin C (ascorbic acid). While, the percentages of DPPH inhibition of the three plants; *I. tricolor*, *P. aviculare* and *R. communis* were noticed to be more or less similar.

### Future prospective

The examined plants will be a better option for the development of new medications due to the vital functions of Ipomoea and Achillea species. Our current research makes it easier to conduct additional research that could result in the identification of new bioactive natural products from these intriguing plants.

The study thoroughly collects the relationships between the phytochemicals of each plant and their associated cytotoxic and antioxidant activities. As a result, it should serve as a suitable work for future research into the phytochemical profiling of the plant and, generally speaking, pharmacological evaluation.

After potatoes, rice, wheat, and maize, sweet potatoes are currently the sixth most important crop in the world, producing over 105 million tons annually (78) (Wang *et al.*, 2016). The roots, vines, and leaves of sweet potatoes are among the many parts that can be eaten. Sweet potato storage roots are rich in dietary fiber, protein, starch, and micronutrients (such as vitamins, minerals, and bioactive compounds), which can supply enough nutrients and energy for good health. The use of heavenly blue (*Ipomoea tricolor*) and sweet potato (*Ipomoea batatas*) as anti-inflammatory agents in traditional medicine is further supported by this study in addition to previous findings.

### Conflicts of Interest

The authors declare no conflict of interest.

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