



# Anticancer and antioxidant activities of ethanolic extract and semi-purified fractions from guava and mango seeds

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## Abstract

Mango (*Mangifera indica*) and guava (*Psidium guajava*) seeds have several pharmaceutical applications and biological activities because as they have been recognized with different bioactive molecules (phenolic compounds) such as flavonoids, phenolic acids, and catechins, so they have antioxidant and anticancer activities. The aim of the present study was to assess in vitro antioxidant and anticancer activities of successive extracts and semi-purified fractions from mango seeds. In this work, mango and guava seeds were collected and extracted using two solvents (ethanol 70% and ethyl acetate) followed by phytochemical screening and determination of biological activities such as antioxidant activity using five assays (DPPH, ABTS, KMnO<sub>4</sub>, Methylene blue and H<sub>2</sub>O<sub>2</sub>) additionally the antiradical activity and hybrid reaction for ethanolic extract of mango seeds as promising extract. The total phenolic, flavonoid, and catechin compounds were determined for all successive extracts, and finally, the anticancer activity of extracts was evaluated using MTT assay against HepG2 cell line and phenolic compounds were identified by HPLC. The phytochemical screening and TLC showed the primary investigation for phenolic compounds of ethanol extracts of both kind of seeds and only ethyl acetate of guava extract as promising extracts. However, HPLC determination of these three extracts showed high amount of gallic acid, naringenin, ellagic acid, and ferulic acid as they have anticancer and antioxidant activities. The antioxidant tests showed that the ethanolic mango extract is the highest antioxidant extract against DPPH by 84.0%, but recorded 82.0% with methylene blue and ABTS assays when compared with ascorbic acid. The ethyl acetate of guava extract showed strong cytotoxic effect with IC<sub>50</sub> 75.5 µg/mL against HepG2 cell line in all tested concentrations. From the obtained results, it could be concluded that mango ethanolic extract and its fractions are the most promising as antioxidants and ethyl acetate of guava extract the most promising in the anticancer activity.

**Keywords** Mango and guava seeds · Chemical constituents · Biological activities · HPLC

## 1 Introduction

Cancer is an uncontrolled growth of mutated and abnormal cells. It is a more complicated disease as it can elevate and grow rapidly by different processes which include cancer resistance to apoptosis, stimulation normal cell signaling, cancer invasion, metastasis, and angiogenesis [1]. According to 2018 data of global cancer (GLOBOCAN), there are expectation about the increase of new cancer cases to exceed

into 18.1 million, and 9.6 million cases of death from cancer. More than 19.3 million cancer new cases with 10 million cancer deaths were estimated by 2020 data of World Health Organization (WHO) as it thought that 30,000,000 cases of cancer will die every year by 2030 [2] and stated that there are some causes that lead to cancer diseases that include pollution, radiation, malnutrition diet, and due to free radicals that result from oxidative stress.

Free radicals are compounds which contain oxygen with an unbalanced number of electrons. When oxygen molecule in the human body splits into single unpaired electron atoms, it becomes unstable and needs to bond with another molecule. As a result, oxidative stress is present [3–5]. Oxidation is a process where oxygen is added or an element becomes more electronegative, or when hydrogen is being removed or an element becomes electropositive [6, 7]. [8] stated that

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in biology, oxidation happens when an element called free radical reacts with oxygen in the body which is a normal process that can aid the body in reducing risk of infections or even help the body to get rid of pathogens.

Free radicals and ROS are produced during normal cellular metabolism, which is also important for cell signaling pathways. Mechanically, mitochondria produce ROS when they produce adenosine triphosphate (ATP), in which case oxygen and electrons combine to form the superoxide anion. Numerous studies have shown that oxidative stress and human pathophysiological disorders may be fundamentally related. Particularly, it is well recognized that oxidative stress affects the DNA molecule, changes signaling pathways, and controls the development of a variety of malignancies, including those of the breast, lung, liver, colon, prostate, ovary, and brain [3]. Antioxidants are any substance that can combat unstable molecules like free radicals is referred to as an antioxidant. Free radicals are countered by antioxidants by yielding some of their own electrons without turning into electron. Antioxidants aid in stopping a chain reaction that may have an impact on other cellular molecules and other cells across the body. As a result, they play a significant role in maintaining health of cells and repairing DNA. Antioxidants can prevent or minimize the damage that free radicals done to cells, as example: reduction of inflammation, lower the risk of brain health problems, and prevent age-related diseases [8].

Types of antioxidants are glutathione and alpha lipoic acid are two effective antioxidants that the human body's cells naturally make. Phytochemicals, which are known as plant chemicals, are substances that are abundant in plants and many of them also appear to have antioxidant characteristics, for example, hesperidin, a phytochemical present in citrus fruits [9].

So much research has been focused on the usage of natural extracts with high antioxidant and anticancer activities for the reduction of cancer and other types of diseases that have been resulted from free radicals. Fruits and vegetables are essential for human nutrition, delivering a substantial proportion of vitamins, minerals, and fibers in our daily diet which characterized based on the antioxidant activity of their phytochemical components, for example, guava fruit and mango fruits [8].

[10] stated that guava fruit is helpful due to its high amount of ascorbic acid, flavonoids, polyphenols, carotenoids, and anthocyanins which make it rich with antioxidants. All parts of guava plant (fruits, seeds, roots, and leaves) have the ability to contribute to the treatment of many health problems due to its several biological activities such as anti-diarrheal, antioxidant, antimicrobial, anticancer, antiviral, and anti-inflammatory. The anticancer activity of

guava extracts was evaluated on several human cell lines as prostate, colon, epidermal cancer, and leukemia and showed significant anticancer activity in the reduction of tumor size [10] and this research proved that the antioxidant activity of guava is due to its high polyphenols and antioxidants such as carotenoids and ascorbic acid.

Mango (*Mangifera indica*) has several pharmaceutical applications due to it having been recognized with different bioactive molecules (phenolic compounds) such as flavonoids, phenolic acids, fatty acid terpenoids, and carotenoids. Each part of the mango (peels, leaves, fruit, seeds) is rich with phenolic compounds which make all mango extracts have several biological activities such as anticancer, antioxidant, antimicrobial, and anti-inflammatory activities [11]. Additionally, they stated that the anticancer activity of mango based on mango's bioactive substances has been strongly related to their capacity to inhibit the growth of cancer cells. This ability is most likely due to a reduction in oxidative stress, which may contribute to the initiation of cancer growth. As a result, it has been proposed that antioxidant supplementation may lower breast cancer mortality and recurrence rates. In addition, the results from animal studies have proved that antioxidant molecules have anticancer characteristics.

The current study was designed to determine the phytochemical contents of ethanolic and ethyl acetate extracts of mango and guava plants and to investigate the potential of its successive extracts as an antioxidant, and anticancer extracts.

## 2 Materials and methods

### 2.1 Materials

#### 2.1.1 Chemicals and reagents

Pure hexane, ethanol, ethyl acetate, and methanol were purchased from E. Merck Co. (Darmstadt, Germany). Sulfarhodamine, 2, 2 diphenyl-1-picrylhydrazyl (DPPH), potassium permanganate ( $\text{KMnO}_4$ ), hydrogen peroxide, 2, 2'-azino-bis(ethylbenzthiazoline-6-sulfonic acid (ABTS+), and ascorbic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ascorbic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA). Ten percent fetal bovine serum (FBS), L-glutamine, containing 100 units/mL penicillin G sodium, streptomycin sulphate, and amphotericin B were all purchased from Lonza (Basel, Switzerland). MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) was obtained from Merck KGaA (Darmstadt, Germany).

## 2.2 Methods

### 2.2.1 Extraction of active ingredients

Guava and mango fruits were collected from local market, Giza, Egypt, during spring 2022. The seeds of both were extracted, air-dried, and then grinded to fine powder. The dried powder (50 g) was subjected to successive extraction with ethyl acetate and ethanol 70% solvents according to Rosenthaler [12].

### 2.2.2 Phytochemical screening

The different extracts were subjected to preliminary phytochemical screening including testing for tannins, sterols, flavonoids, glycosides, and reducing sugar as the following.

#### a. Ferric chloride test for phenolic compounds

Phenolics were detected using methods of [13]. Positive result was a dark green/bluish-black color.

#### b. Test for glycosides and/or carbohydrates

Extracts were evaluated for carbohydrates and reducing sugars in the usual manner using Molishs and Fehling reagents following Harper [14].

#### iii. Test for proteins and amino acids

Proteins were determined using methods of [13]. A positive result was the pink-colored solution (in the ethanolic layer).

#### iv. Test for flavonoids

The test was conducted by adding concentrated HCl drop wise to 1 mL of solution containing a fragment of magnesium ribbon according to [13], a positive result gave pinkish color.

#### e. Anthocyanins

Detection of anthocyanins was done using the method of [13].

#### f. Coumarin

Detection of coumarin was done using the method of [13].

#### g. Wagner's test for alkaloids

Alkaloids were detected using methods of [13]. Positive result was brown/reddish precipitate.

#### h. Antioxidant activity

Detection of  $\text{KMnO}_4$  (non-radical) activity was done using method of [15].

Detection of DPPH (radical) activity was done using method of [16].

## 2.3 Thin layer chromatography (TLC) bio-autography for antioxidant activity

The separation of active compounds from ethanol and ethyl acetate extracts of mango and guava was performed using precoated silica gel plates (TLC F254) with hexane: acetone at ratio (9:1 v/v) systems as mobile phase. A rapid TLC screening method for antioxidant activity was done using radical DPPH as a spray reagent. TLC was performed for –ethanol and ethyl acetate extracts of mango and guava extracts as described earlier by Nair et al. [17]. The plates were dried, and antioxidant activity was detected by spraying DPPH in methanol onto TLC plates. The development of yellow or white spots against a purple background indicated the presence of antioxidant compounds.

## 2.4 Compounds determination

### 2.4.1 Determination of total phenolic compounds

Total phenolic compounds were determined using the method described by Meda et al. [18]. The absorbance was measured at 750 nm using a spectrophotometer. Phenolic contents were calculated based on the standard curve of gallic acid used as standard.

### 2.4.2 Determination of total flavonoids

Flavonoid compounds were determined using the method described by Zhishen et al. [19]. The absorbance was measured spectrophotometrically against a blank at 415 nm. Quercetin was served as the standard compound for the preparation of the calibration curve.

### 2.4.3 Determination of total catechins

Condensed tannins of seed extracts were determined using the vanillin assay described by Khlifi et al. [20] with some modifications. To 50 mL of the extract, 1.5 mL of vanillin/methanol (4%) solution was added and mixed. Then, 1 mL of concentrated HCl was added and allowed to react at room temperature for 1 h. The absorbance was measured against a blank at 550 nm. The total concentration of condensed catechins was expressed in micrograms of catechin equivalents

per milligram dry matter with reference to the catechin calibration curve.

#### 2.4.4 Ascorbic acid determination

DCPIP and standard AA solutions 200 mg of DCPIP, 106 mg of  $K_2HPO_4$ , and 90 mg of  $NaH_2PO_4$  were dissolved in 1000 mL of double distilled water. This solution was stable for 30 days and calibrated by a standard AA solution before use for titrations. Titration Procedure DCPIP not only acts as an oxidant but also functions as a visual acid–base indicator for the detection of the end point of titration, where the pink color is observed in the solution, which is generated by the excess of DCPIP reagent (Eq. 2). The definite volume of vitamin C containing solution was transferred into the conical flask. If mentioned volume is less than 10 mL, acid acetic 3% (v/v) was added up to total volume of 10 mL, and then titrated against the calibrated DCPIP solution up to equivalent point. The above procedure was repeated four times for each sample [21].

## 2.5 Biological activities

### 2.5.1 DPPH radical scavenging activity

The scavenging effects of successive extracts from ethanol and ethyl acetate extracts of mango and guava seeds were determined by the method of [16], where 1.0 mL of 0.16 mM DPPH solution (in methanol) was added to a test tube containing 1.0 mL aliquot of sample at around

100 ppm. The absorbance of all the sample solutions, ascorbic acid as antioxidant standard, was measured at 517 nm. The percentage (%) of scavenging activity was calculated as the following: % Antioxidant activity = (Control – Sample  $\times$  100) / Control, where: control in DPPH solution (0.16 mM).

### 2.5.2 ABTS radical cation scavenging assay

This assay was based on the ability of different substances to scavenge (2, 2'-azino-bis ethylbenzthiazoline-6-sulfonic acid (ABTS<sup>+</sup>)) radical cation in comparison to a standard (BHT). According to Re et al. [22], the photometric assay was conducted on 1 mL of (ABTS<sup>+</sup>) and 1 mL of tested samples and the measurements were taken at 734 nm after 1 min. The antioxidant activity of the tested samples was calculated by determining the decrease in absorbance at different concentrations by using the following equation:  $E = ((Ac - At) / Ac) \times 100$ , where  $At$  and  $Ac$  are the respective absorbance of tested samples and ABTS<sup>+</sup>.

### 2.5.3 $KMnO_4$ as non-radical scavenging activity

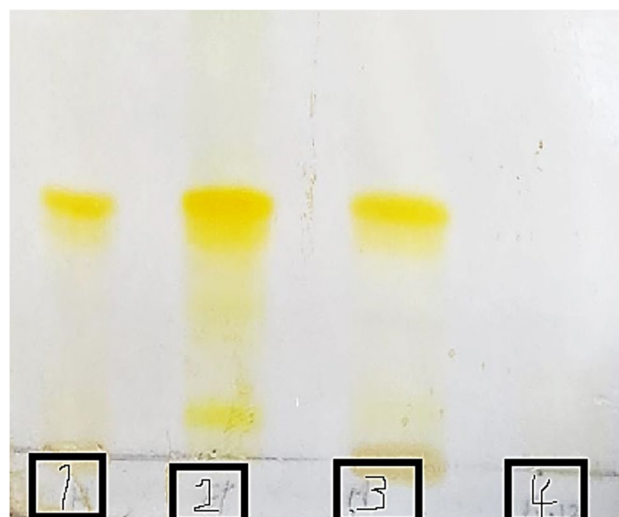
The test was carried out by adding 1 mL of potassium permanganate solution to 1 mL sample extract and stand for 5 min. Positive result converts pinkish color to colorless.

The scavenging effects of successive ethanol and ethyl acetate extracts from mango and guava were determined by the method of [15], where 1.0 mL of 0.16 mM  $KMnO_4$

**Table 1** Phytochemical screening of successive extracts from guava and mango seeds

Phytochemical com	Guava seeds		Mango seeds	
	Ethyl acetate extract	Ethanol 70% extract	Ethyl acetate extract	Ethanol 70% extract
Phenolic	-	+	++	+++
Carbohydrates	-	-	+	+
Proteins	-	-	-	-
Fatty acids	-	-	-	-
Flavonoids	-	-	+	+
Anthocyanins	-	-	-	-
Coumarins	-	-	+	+
Glycosides	+	-	+	-
Alkaloids	-	+	-	+
Antioxidant by $KMnO_4$	-	++	+++	+++
Antioxidant by DPPH	-	+++	+++	+++

+, present; -, absent



**Fig. 1** TLC of successive mango and guava extracts: (1) ethyl acetate extract of mango seeds, (2) ethanol extract of mango seeds, (3) ethanol extract of guava seeds, (4) ethyl acetate extract of guava seeds

**Table 2** Total phenolic, flavonoids, catechins (as mg/100 g), and ascorbic acid of content (as µg/mL) in mango and guava successive extracts

Extract	Mango		Guava	
	Ethanol	Ethyl acetate	Ethanol	Ethyl acetate
Phenolic	138.16 ± 6.91	543.6 ± 27.18	69.41 ± 3.47	77.03 ± 3.85
Flavonoids	19.61 ± 0.98	23.78 ± 1.19	10.26 ± 0.51	11.75 ± 0.59
Catechins	5.65 ± 0.28	11.25 ± 0.56	1.60 ± 0.08	8.997 ± 0.45
Ascorbic acid	1274.91 ± 63.75	ND	242.8 ± 12.14	ND

Values are expressed as mean ± SD. ND, not detected

solution (in dist. water) was added to a test tube containing 1.0 mL aliquot of sample at around 100 ppm. The absorbance of all the sample solutions, ascorbic acid as antioxidant standard, was measured at 514 nm. The percentage (%) of scavenging activity was calculated as the following: % Antioxidant activity = (Control – Sample × 100) / Control, where control is potassium permanganate solution (0.16 mM).

**2.5.4 Methylene blue method**

The scavenging effects of crude extract were the following where 1.0 mL of 0.002 M methylene blue solution (in water) was added to a test tube containing 1.0 mL aliquot of sample. The mixture was vortexed for 1 min and kept at room temperature for 30 min in the dark. The absorbances of all the sample solutions and ascorbic acid as natural standard were measured at 663 nm. The percentage (%) of scavenging activity was calculated as the following: % Antioxidant activity = (Control – Sample × 100) / Control where control is methylene blue solution [15].

**2.5.5 Hydrogen peroxide scavenging assay**

The ability of the extract to scavenge hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was determined according to the method of Ruch et al. [23]. The absorbance was measured at 230 nm. Ascorbic acid was used as the positive control. The ability of the extracts to scavenge the H<sub>2</sub>O<sub>2</sub> was calculated using the following equation:

$$H_2O_2 \text{ scavenging activity percentage} = [(A0 - A1)/A0] \times 100$$

where:

A0 absorbance of control.

A1 absorbance of sample.

**2.5.6 Blending of seeds extract and ascorbic acid as natural standard (hybrid drug)**

The ethanol extract of mango seed as a promising extract was used for the determination of its antioxidant activity using DPPH method (as mentioned before) after blending with natural antioxidant standard (BHT) at 100 µg/mL as described by Shalaby and Shanab [24].

**2.5.7 Antiradical activity for promising extract**

DPPH• radicals in their radical form have characteristic absorbance at 517 nm, which disappears after its reduction by an antiradical compound. The reduction of DPPH can thus be monitored by measuring the decrease in its absorbance at 517 nm when react with seed extracts or its fractions during the reaction time (10–110 s) at 100 µg mL<sup>-1</sup>. All details related to the method are described by Shalaby and Shanab [24]. The antiradical

**Table 3** Antioxidant activity (%) of successive extracts of mango and guava seeds against KMnO<sub>4</sub>, DPPH, methylene blue, ABTS, and H<sub>2</sub>O<sub>2</sub> assay at 100 µg mL<sup>-1</sup>

Extract	Guava seeds		Mango seeds		Standard
	Ethyl acetate	Ethanol 70%	Ethyl acetate	Ethanol 70%	Ascorbic acid
DPPH	6.95 ± 0.35	58.0 ± 2.9	61.0 ± 3.05	84.0 ± 4.2	88.5 ± 4.43
ABTS	55.0 ± 2.75	76.0 ± 3.8	73.0 ± 3.65	82.0 ± 4.1	93.67 ± 4.68
KMnO <sub>4</sub>	5.0 ± 0.25	33.56 ± 1.68	77.6 ± 3.88	7.65 ± 0.38	53.7 ± 2.69
Methylene blue	81.0 ± 4.05	27.6 ± 1.38	37.0 ± 1.85	82.0 ± 4.1	72.51 ± 3.63
H <sub>2</sub> O <sub>2</sub>	51.7 ± 2.59	17.1 ± 0.86	17.0 ± 0.85	17.0 ± 0.85	50.12 ± 2.51

Values are expressed as mean ± SD

**Table 4** Hybrid reaction for antioxidant activity (%) of mango ethanolic extract against DPPH radical assay

Sample	Antioxidant activity (%)
100% extract	62.4 ± 3.12
80% extract + 20% ascorbic acid	56.4 ± 2.82
60% extract + 40% ascorbic acid	49.6 ± 2.48
40% extract + 60% ascorbic acid	65.5 ± 3.28
20% extract + 80% ascorbic acid	81 ± 4.05
100% ascorbic acid	87 ± 4.35

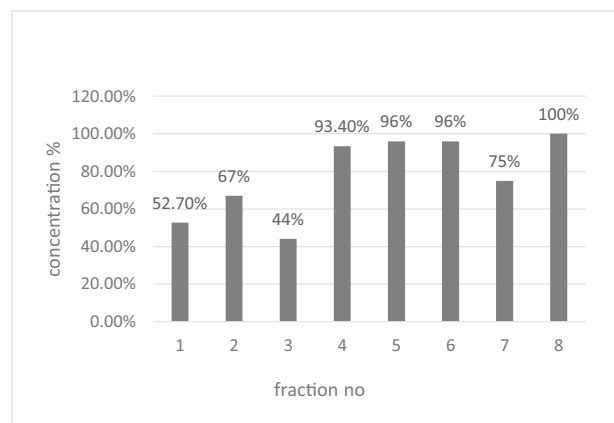
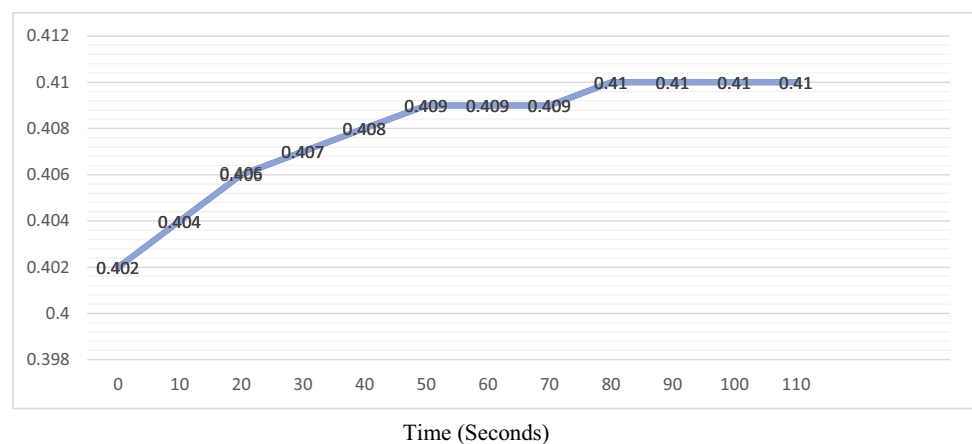
Values are expressed as mean ± SD

activity (AU515) was calculated according to the equation:  $AU515 = (A0 - A1) - (A0K - A1K)$ , where AU515 is the antiradical activity of the extract, A0K the absorbance of the control sample at the beginning of the reaction, and A1K the absorbance of the control sample after incubation times (10–110 s) of the reaction. Because  $A0K - A1K$  was always equal to 0, the above equation was simplified to:  $AU515 = A0 - A1$ .

### 2.5.8 Fractionation of ethanolic seeds extract as promising extract

The chromatographic column (40 cm length, 2.5 cm diameter) was packed with 150 g silica gel (60–120 mesh for column chromatography) using hexane as solvent. A total of 5.0 g of ethanol crude extract of mango (as promising crud extract) were grounded very well with silica gel powder and then placed on the top of the packed column. The column was then sequentially eluted with 100% hexane followed by ethyl acetate and ethanol solvent, the polarity increased by 15% between each mobile phase mixtures (total 8 fractions were obtained) as the following:

**Fig. 2** Antiradical unit (AU) of mango ethanolic extract against DPPH assay



**Fig. 3** Antioxidant activity (%) of semi-purified fractions of mango ethanolic extract against ABTS at 100 µg/mL

Solvent/sample no	Fractions no							
	1	2	3	4	5	6	7	8
Hexane (mL)	100	70	40	10	0	0	0	0
Ethyl acetate (mL)	0	30	60	90	100	70	40	100
Ethanol (mL)	0	0	0	0	0	30	60	0

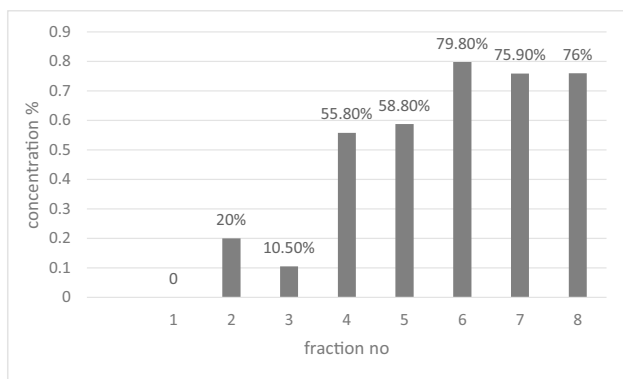
### 2.6 Anticancer activity by MTT assay

#### 2.6.1 Cells

Human epithelial-like hepatocellular carcinoma (HepG2) was purchased from ATCC, USA and used in the study.

#### 2.6.2 Cell culture

The cells were routinely cultured in DMEM. It is supplemented with 10% FBS, 2 mM L-glutamine, containing 100 units/mL penicillin G sodium, 100 units/mL streptomycin



**Fig. 4** Antioxidant activity (%) of semi-purified fractions of mango ethanolic extract against methylene blue at 100 µg/mL

sulphate, and 250 ng/mL amphotericin B, all from Lonza (Basel, Switzerland). Cells were maintained at sub-confluency at 37 °C in humidified air containing 5% CO<sub>2</sub>. For sub-culturing, monolayer cells were harvested after trypsin/EDTA treatment at 37 °C. Cells were used when confluence had reached 75%.

### 2.6.3 MTT cytotoxicity

The MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) was obtained from Merck KGaA (Darmstadt, Germany). It is used to assess the cytotoxicity of the tested samples. It is based on the capability of active mitochondrial dehydrogenase enzyme of living cells to cleave the tetrazolium rings of the yellow MTT and form dark blue insoluble formazan crystals. The crystals are solubilized leading to liberation of dark blue color directly proportional to the number of live cells. Briefly, cells (1 × 10<sup>4</sup> cells/well) were seeded in serum-free media in a flat bottom 96-well microplate and treated with 20 µl of different concentrations of the tested samples for 24 h at 37 °C, in a humidified 5% CO<sub>2</sub> atmosphere. After incubation, media were removed and 40 µl MTT solution/well were added and incubated for

an additional 4 h. MTT crystals were solubilized by adding 180 µl of acidified isopropanol/well and the plates were shaken at room temperature, followed by photometric determination of the absorbance at 570 nm using microplate ELISA reader (FLUOstar OPTIMA, BMG LABTECH GmbH, Ortenberg, Germany). Three time repeats were performed for and the average was calculated according to Hansen et al. [25].

### 2.6.4 HPLC conditions

HPLC analysis was carried out using an Agilent 1260 series. The separation was conducted using Eclipse C18 column (4.6 mm × 250 mm i.d., 5 µm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate of 0.9 mL/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–5 min (80% A); 5–8 min (60% A); 8–12 min (60% A); 12–15 min (82% A); 15–16 min (82% A); and 16–20 (82% A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 5 µl for each of the sample solutions. The column temperature was maintained at 40 °C.

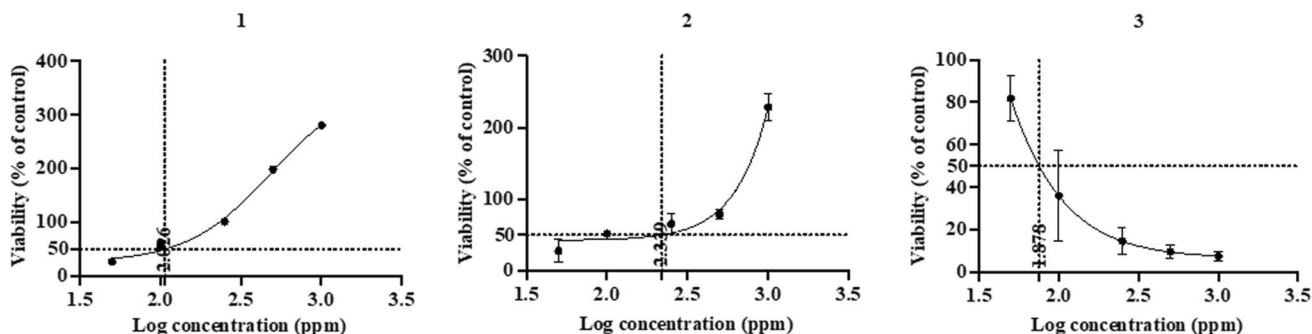
## 2.7 Statistical analysis

All the data are expressed as mean ± standard deviation. Statistical comparison was performed via a one-way analysis of variance followed by Duncan's multiple range test (DMRT). *P*-values of less than 0.05 (*P* < 0.05) were considered as significant.

## 3 Results and discussion

### 3.1 Phytochemical screening

Mango and guava are considered as medicinal plants due to having a lot of secondary metabolites such as phenolic



**Fig. 5** The cytotoxic effect of (1) mango ethyl acetate extract, (2) mango ethanol extract, and (3) guava ethanol extract on HepG2 cell line

**Table 5** IC<sub>50</sub> of the promising extracts of mango and guava on HepG2 cell line

Sample no	Sample name	IC <sub>50</sub> (ppm ± SE)
1	Mango ethyl acetate extract	106.25 ± 1.64
2	Mango ethanol extract	218.13 ± 43.4
3	Guava ethanol extract	75.47 ± 20.8

compounds, flavonoids, alkaloids, plant acids, and glycosides making them contribute to several biological activities. The precursory qualitative phytochemical screening for the secondary metabolites of successive extracts of mango and guava seeds is detected in Table 1. Mango seeds successive extracts showed high secondary metabolites of phenolics, flavonoids, coumarins, and alkaloids in addition to antioxidant activity against KMnO<sub>4</sub> and DPPH agreeing with [26] who proved that mango seeds obtained high amount of phenolics and high antioxidant activity against KMnO<sub>4</sub> and DPPH. However, in guava seeds successive extracts, ethanol 70% extract contained phenolics, alkaloids, and antioxidant activity against KMnO<sub>4</sub> and DPPH unlike ethyl acetate extract.

To prevent the oxidative damage that leads to disease, antioxidants are a class of chemical substances that have the ability to reduce the cell damage caused by free radicals by quickly supplying electrons and converting the free radicals into stable forms [27]. Antioxidants, also referred to as bioactive chemicals, are present in plants as a pest and disease defense mechanism. The DPPH method was used in this study's quantitative phytochemical analysis to assess the quantity of phenolic compounds and antioxidants level and HPLC was used to assess the content of these phenolic chemicals [27]. These results agreed with previous data obtained by [28] as they reported that presence of polyphenolic compounds as phenols, flavonoids, fatty acids, and essential oil in guava can contribute to several biological activities with therapeutic effect as antioxidant and anticancer activities.

### 3.2 TLC bio-autography for antioxidant activity

Separation of mango and guava seeds successive extracts by TLC bio-autography showed that all successive extracts of both mango and guava seeds produced yellowish and white color on the purple background were considered as strong antioxidants as shown in Fig. 1. However, guava ethyl acetate extracts only contained a little amount of antioxidant compounds that is may be because they need to change and use other solvents.

DPPH reagent is used to measure the scavenging activity of free radicals and the electron-donating activity and evaluate the antioxidant activity of several compounds [29].

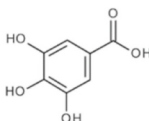
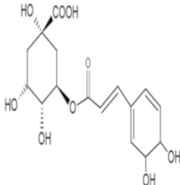
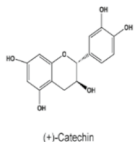
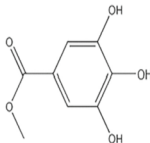
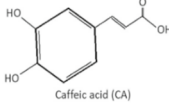
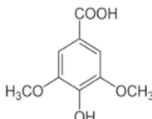
### 3.3 Compound determination of mango and guava successive extracts (phenolic, flavonoids, catechins, and ascorbic acid)

Mango and guava have a lot of compounds that are important for human health due to their antioxidant activities such as phenolic, flavonoids, and catechins. Hence, the total concentration of these compounds was evaluated and the results in Table 2 revealed that both mango and guava seeds contain higher concentrations of phenolics, flavonoids, and catechins in their ethyl acetate extracts than ethanolic extracts. However, mango seeds ethyl acetate extract was found to have higher phenolics, flavonoids, and catechins ( $543.6 \pm 27.18$ ,  $23.78 \pm 1.19$ ,  $11.25 \pm 0.56$  mg/100 g, respectively) than guava seeds ethyl acetate extracts ( $77.03 \pm 3.85$ ,  $11.75 \pm 0.59$ ,  $8.997 \pm 0.45$  mg/100 g, respectively). Moreover, mango seeds also contain higher concentrations of these compounds in ethanolic extracts than guava seeds ethanolic extracts. These results were contaminant with those of [26] who proved that mango seeds obtained high amount of total phenolics (153 mg/g DW) reflecting its high antioxidant activity against KMnO<sub>4</sub> and DPPH in addition to the high antioxidant activity of guava ethanolic extract.

Plant polyphenols can function as reducing agents, hydrogen atom donor, and a singlet oxygen scavenger. Some of the most significant and physiologically active chemicals are phenolic compounds. Flavonoids are another phenolic chemical family found in fruits and vegetables with increased antioxidant activity [30]. The presence of high phenolic, flavonoid, and catechin compounds in plants refer to the fact that these plants have therapeutic activities as antioxidant and anticancer activities which agreed with previous studies obtained by [31].

Ascorbic acid is an antioxidant found in many plants according to [32], so it was detected in our samples and was found to be much higher in mango ethanolic extract than guava ethanolic extract ( $1274.91 \pm 63.75$ ,  $242.8 \pm 12.14$  ppm) respectively making mango a high antioxidant plant because ascorbic acid not only binds with free radicals but also increases the content of crops for more helpful impacts on human health and contributes in the treatment of many diseases as stated by [33]. These results were like a previous study by [1] who found the concentration of ascorbic acid in mango peels, mango kernels, and guava seeds as  $0.086 \pm 0.01\%$ ,  $0.085 \pm 0.01\%$ ,  $0.034 \pm 0.03\%$ , respectively.

**Table 6** List of phytochemical constituents (as  $\mu\text{g/mL}$ ) in ethyl acetate extract of mango seeds analyzed by HPLC

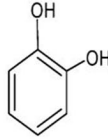
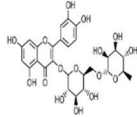
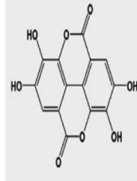
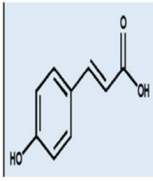
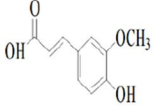
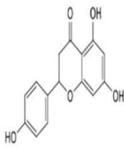
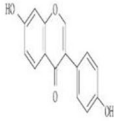
S.No	Retention time (min)	Chemical name	Chemical structure	Conc. ( $\mu\text{g/ml}$ )	Biological activities	References
1	3.266	Gallic acid		2334.67	Antioxidant Anticancer Antiinflammation	[38]
2	4.053	Chlorogenic acid		5.91	Antioxidant Anti-inflammatory Antibacterial Antipyretic Antiviral Anti-obesity Anti-microbial Anti-hypertension Hepatoprotective Neuroprotective Cardioprotective Free radicals' scavenger	[42]
3	4.390	Catechin		50.49	Anticancer Antiviral Anti-obesity Antioxidant Anti-cardiovascular Anti-inflammatory Neuroprotective	[43]
4	5.518	Methyl gallate		17.73	Anticancer Antimicrobial Anti-inflammatory	[44]
5	6.011	Caffeic acid		0.07	Antioxidant Anti-inflammatory Antimicrobial	[45]
6	6.517	Syringic acid		45.73	Antioxidant Antimicrobial Anti-inflammatory	[46]

### 3.4 Antioxidant activity for mango and guava seeds successive extracts

Antioxidant activity of mango and guava successive extracts was detected against radical assays (DPPH and ABTS) and non-radical assays ( $\text{KMnO}_4$ , methylene blue, and  $\text{H}_2\text{O}_2$ ) as recorded in Table 3. Regarding mango seeds, our results revealed that ethanolic extract had the highest antioxidant activity against DPPH followed by ABTS and methylene blue ( $84.0 \pm 4.2$ ,  $82.0 \pm 4.1$ ,  $82.0 \pm 4.1\%$ ) respectively compared to the standard ascorbic acid ( $88.5 \pm 4.43$ ,  $93.67 \pm 4.68$ ,  $72.51 \pm 3.63\%$ ), respectively. Furthermore, mango ethanolic extract antioxidant activity against methylene was even higher than the standard ascorbic acid ( $82.0 \pm 4.1$ ,  $72.51 \pm 3.63\%$ ), respectively.

Highest antioxidant activity in guava seeds was found in ethanolic extract against ABTS and DPPH followed by  $\text{KMnO}_4$  ( $76 \pm 3.8$ ,  $58 \pm 2.9$ ,  $33.56 \pm 1.68\%$ ) respectively in comparison to those of ascorbic acid ( $93.67 \pm 4.68$ ,  $88.5 \pm 4.43$ ,  $53.7 \pm 2.69\%$ ), respectively. Mango and guava ethanolic extracts had higher antioxidant activities against ABTS and DPPH compared to  $\text{KMnO}_4$  and  $\text{H}_2\text{O}_2$  agreeing with the findings of [15] who stated that this may be due to that ABTS and DPPH are unstable and radical assays that can react faster with several antioxidants, but non-radical assays are stable and require more time when react with antioxidants. However, guava ethyl acetate extract had the highest antioxidant activity against methylene blue and  $\text{H}_2\text{O}_2$  ( $81 \pm 4.05$ ,  $51.7 \pm 2.59\%$ ) respectively being higher than those of the standard ascorbic acid

Table 6 (continued)

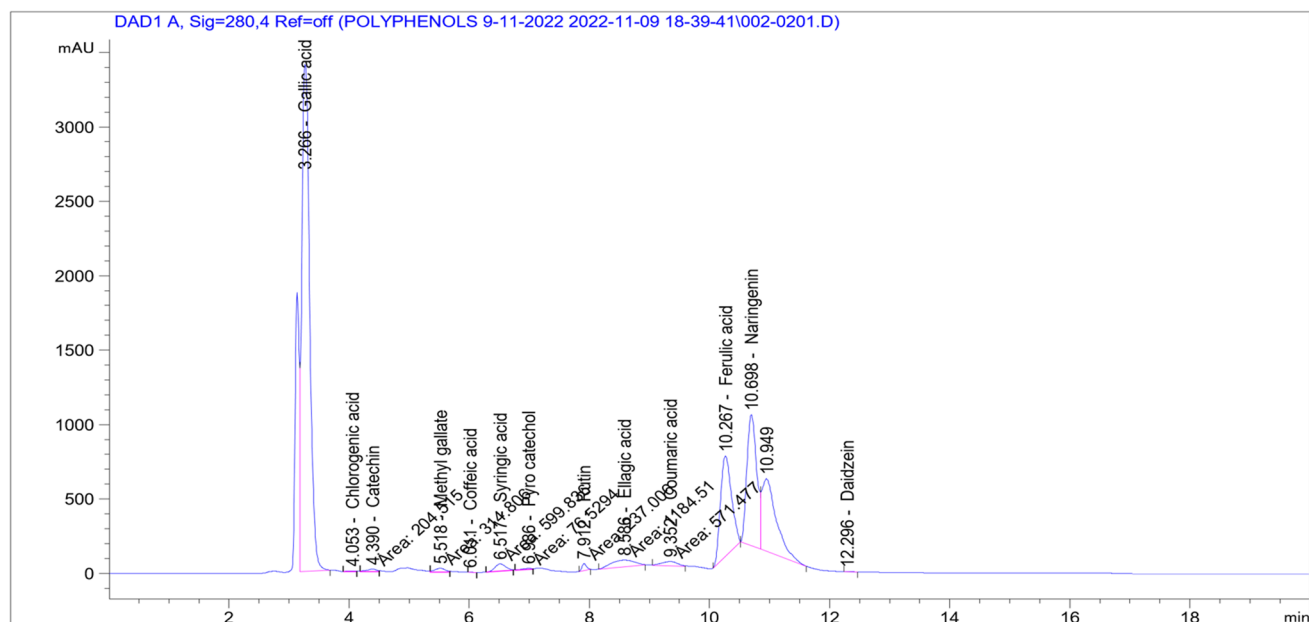
7	6.986	Pyro catechol		10.33	Antibacterial Antimicrobial Antifungal	[46]
8	7.912	Rutin		28.01	Anti-inflammatory immunomodulator Antimicrobial Anticancer	[47]
9	8.586	Ellagic acid		309.01	Anti-atherogenic Antitumor Anti-inflammatory Neuroprotective Anticancer	[48]
10	9.352	Coumaric acid		14.71	Anti-inflammatory Antioxidant Antimicrobial Anticancer	[49]
11	10.267	Ferulic acid		448.82	Anti-inflammatory Antioxidant Antidiabetic Anticancer	[50]
12	10.698	Naringenin		954.51	Antioxidant Anti-inflammatory Anti-mutagenic Anticancer Antimicrobial	[40] [48]
13	12.296	Daidzein		0.32	Antioxidant Anti-inflammatory Anticancer	[51]

( $72.51 \pm 3.63$ ,  $50.12 \pm 2.51\%$ ), respectively. These findings agreed with [26] who found that mango seeds obtained high antioxidant activity against  $\text{KMnO}_4$  and DPPH in addition to the high antioxidant activity of guava ethanolic extract.

### 3.5 Hybrid reaction for promising mango ethanolic extract

The hybrid reaction was used to detect the antioxidant activity of the promising mango ethanolic extract combined with the standard antioxidant, ascorbic acid (vitamin C) against DPPH at 100  $\mu\text{g}/\text{mL}$ . The results shown in

Table 4 concluded that the sample containing (20% ethanolic mango extract: 80% ascorbic acid) had the highest antioxidant activity against DPPH among other samples compared to 100% ascorbic acid ( $81 \pm 4.05$ ,  $87 \pm 4.35\%$ ), respectively. The results showed that the addition of ascorbic acid in a ratio of 20 to 80% extract and in 40% ascorbic acid to 60% extract went against the antioxidant activity and lower its percentage while the addition of it in ratio of 60% and 80% to 40% and 20% respectively of extract show higher level of antioxidant activity, which evaluated the high antioxidant activity of extract due to its effect on ascorbic acid (antioxidant molecule) [33]. The current work study is like the studies of [34] who



**Fig. 6** HPLC chromatogram of ethyl acetate extract from mango seeds

reported that the DPPH free radical scavenging ability on mango and guava fruit pulps at different maturation stages using standard ascorbic acid for comparison. The scavenging ability of an antioxidant is measured by its ability to quench the stable DPPH radical with maximum absorption at 517 nm. The findings demonstrated that mango and guava fruit pulps were effective radical scavengers at various stages of fruit ripeness, and a strong correlation was found between these fruit pulps' scavenging capacities and ascorbic acid concentrations. With increasing concentration, the fruits became more effective at scavenging. Finally, the antioxidant activity of mango ethanolic extract combined with ascorbic acid was not higher than antioxidant activity of ascorbic acid only, so mango ethanolic extract cannot be used as an antioxidant because it lowered the standard activity.

### 3.6 Antiradical test for promising extract

Mango ethanolic extract was the most promising extract as shown in Table 2 so it has been followed by antiradical test against DPPH at 100 g/mL for 10–110 s. The obtained results showed that the antiradical activity of mango seeds ethanolic extract against DPPH increased with time then became constant after 80 s; antiradical unit (AU) increased from 0.402 to 0.41 (Fig. 2).

Mango ethanolic extract may have a good antiradical activity with the potential of obtaining the free radicals due to the increase of its AU against DPPH by time. However, these results disagreed with those of [35] who stated that

DPPH radical scavenging activity of hexane extract was higher than that of ethanol extract because hexane extracts have more antioxidant components soluble in oil, such as  $\beta$ -carotene and vitamin E (tocopherols and tocotrienols).

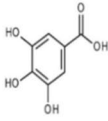
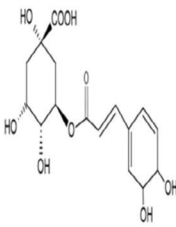
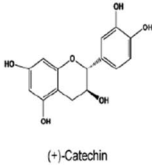
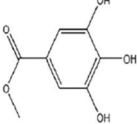
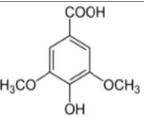
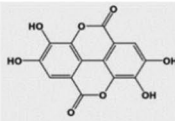
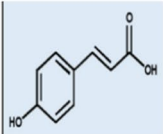
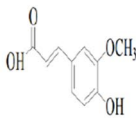
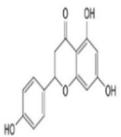
### 3.7 Antioxidant activity of semi-purified fractions

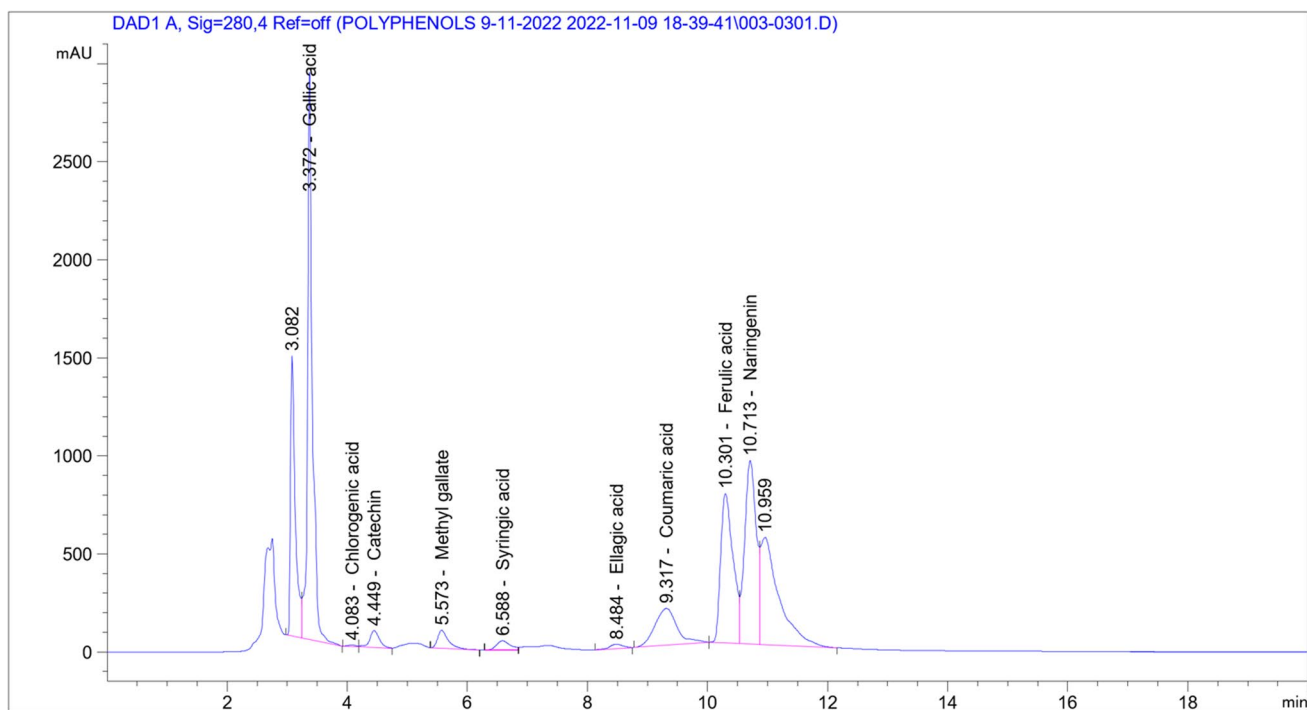
Semi-purified fractionation was performed only for promising extract (mango seeds ethanolic extract) and 8 fractions were obtained and evaluated for their antioxidant activity against ABTS as a radical assay and methylene blue as a non-radical assay at 100  $\mu$ g/mL. The obtained results showed that fraction no.8 had the highest antioxidant activity against ABTS after 1 min followed by fraction nos. 6 and 5 equally, then fraction no. 4 (100, 96, 96, 93.40%) respectively while fraction no. 6 had the highest antioxidant activity against methylene blue after 15 min followed by fraction no. 8 then no. 7 (79.80, 76, 75.90%) respectively as shown in Figs. 3 and 4. So, fraction no. 8 and fraction no. 6 were more promising to achieve a high potential impact as an antioxidant against free radical molecules.

### 3.8 Anticancer activity

The potential anticancer activity of mango ethyl acetate and ethanolic extracts in addition to guava ethanolic extract was demonstrated using MTT cytotoxic assay against HepG2 cell line and the results are recorded in Fig. 5 and Table 5. Results revealed different effects of each sample on HepG2 cell line. Samples 1 and 2 increased the proliferation of the samples

**Table 7** List of phytochemical constituents (as  $\mu\text{g/g}$ ) in ethanol extract of mango seeds analyzed by HPLC

S.No	Retention time (min)	Chemical name	Chemical structure	Conc ( $\mu\text{g/ml}$ )	Biological activities	References
1	3.372	Gallic acid		1238.06	Antioxidant Anticancer Antiinflammation	[38]
2	4.083	Chlorogenic acid		12.92	Antioxidant Anti-inflammatory Antibacterial Antiviral Anti-obesity Anti-microbial Anti-hypertension Hepatoprotective Neuroprotective Cardioprotective Free radicals' scavenger	[42]
3	4.449	Catechin	 (+)-Catechin	235.30	Anticancer Antiviral Anti-obesity Antioxidant Anti-cardiovascular Anti-inflammatory Neuroprotective	[43]
4	5.573	Methyl gallate		62.52	Anticancer Antimicrobial Anti-inflammatory	[52]
5	6.588	Syringic acid		57.35	Antioxidant Antimicrobial Anti-inflammatory	[21]
6	8.484	Ellagic acid (phenolic)		82.39	Anti-atherogenic Antitumor Anti-inflammatory Neuroprotective Anticancer	[39]
7	9.317	Coumaric acid		122.67	Anti-inflammatory Antioxidant Antimicrobial Anticancer	[49]
8	10.301	Ferulic acid		575.78	Anti-inflammatory Antioxidant Antidiabetic Anticancer	[50]
9	10.713	Naringenin		1199.24	Hepatoprotective Anti-atherogenic Anti-inflammatory Anti-mutagenic Anticancer Antimicrobial	[40]



**Fig. 7** HPLC chromatogram of ethanol extract from mango seeds

significantly in a dose-dependent manner up to 285% and 220% respectively with  $IC_{50}$  equal to 106.25 and 218.1 ppm, respectively. Sample 3 showed strong cytotoxic effect on the cells in a dose-dependent manner with  $IC_{50}$  75.5 ppm against HepG2 cell line in all tested concentrations.

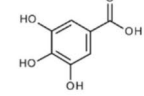
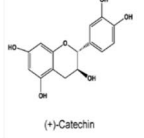
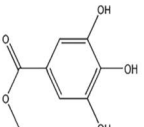
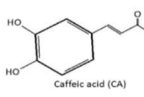
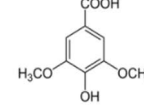
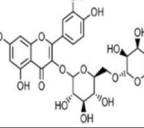

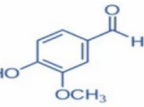
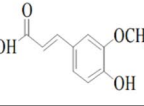
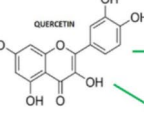
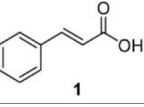
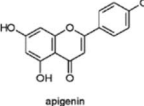
The obtained results stating that ethyl acetate extract of guava seeds had the highest anticancer effect against HepG2 cell line among the other tested samples were similar to a study by [10] where the potential use of guava plant extracts, including leaf oil, leaf extract, and guava seed, in chemotherapy was examined, it was found that the extracts significantly inhibited the growth of numerous human cancer cell lines, including prostate, melanoma, epidermis, colon, and leukemia, and they reported a high reduction in the level of serum of prostate-specific antigen (PSA) and after using budding guava leaves with a dose concentration of 1.5 mg/mouse/day, there was a reduction in the size of the tumor in the xenograft mouse cancer model. Guava leaf essential oil (0.019–4.962 mg/mL) has been shown to be highly effective at inhibiting the growth of murine leukemia and epidermal carcinoma cell lines of human mice (P388) according to [10]. The largest amount of vitamin C in guava is found in its seeds additionally, it contains high antioxidants as carotenoids and polyphenol as the antioxidant content of guava is especially useful for human health as strong anticancer activity compared with other extracts as proved by [36]. The anticancer effect of guava, especially ethyl acetate extract, may be due to its high content of phenols, flavonoids, and catechins as mentioned in Table 2.

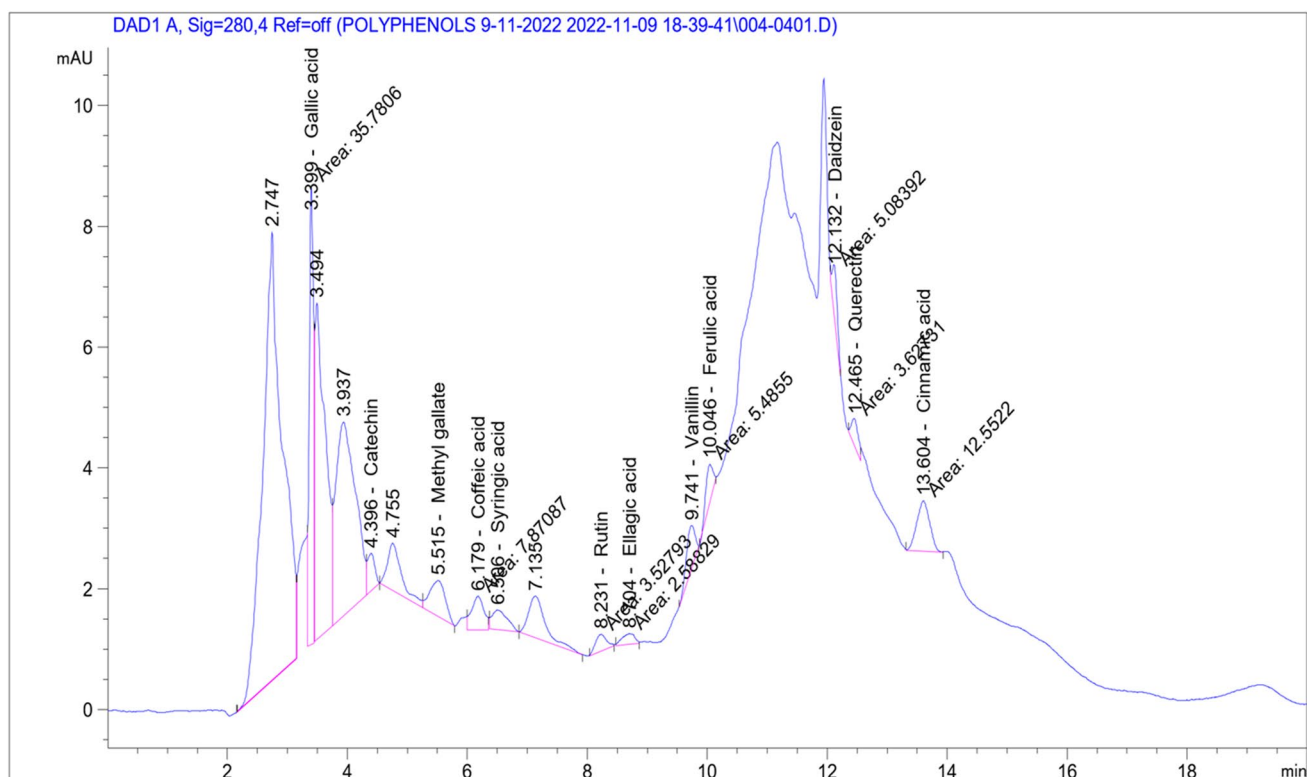
### 3.9 Profile of phenolic compounds quantification by HPLC

Medicinal plants had mixture of natural compounds with several biological activities for human health and HPLC was used to detect these components in mango and guava successive extracts. The results of HPLC showed that mango seeds ethyl acetate extract had high concentrations of gallic acid, naringenin, ellagic acid, and ferulic acid as they have anticancer and antioxidant activity (Table 6 and Fig. 6). The ethanolic extract of mango seeds had high concentration of gallic acid, naringenin, catechins, and ferulic acid as they have anticancer and antioxidant activity (Table 7 and Fig. 7), while guava seeds ethanolic extract had a combination of gallic acid, chlorogenic acid, catechin, methyl gallate, caffeic acid, and syringic acid but with lower concentration than other extracts of mango seeds (Table 8 and Fig. 8). Different compounds were identified and quantified by HPLC technique (Tables 6, 7, and 8).

The obtained results revealed that gallic acid was the major compound in mango seeds ethyl acetate extract represented as 2334.67  $\mu\text{g/mL}$ , followed by naringenin and ferulic acids represented as 954.51 and 448.82  $\mu\text{g/mL}$ , respectively, whereas mango seeds ethanolic extract contained gallic acid and naringenin in concentration of 1238.06 and 1199.24  $\mu\text{g/mL}$ , respectively, showing that mango seed extracts had a wide range of biological activities and can be used as an effective treatment in many cases as antioxidant and anticancer according

**Table 8** List of phytochemical constituents (as  $\mu\text{g/g}$ ) in ethanol extract of guava seeds analyzed by HPLC

S.N o.	Retention time (min)	Chemical name	Chemical structure	Conc. ( $\mu\text{g/ml}$ )	Biological activities	References
1	3.399	Gallic acid		2.68	Antioxidant Anticancer Antiinflammation	[38]
3	4.396	Catechin		1.32	Anticancer Antiviral Anti-obesity Antioxidant Anti-cardiovascular Anti-inflammatory Neuroprotective	[43]
4	5.515	Methyl gallate		0.59	Anticancer Antimicrobial Anti-inflammatory	[25]
5	6.179	Caffeic acid		0.59	Antioxidant Anti-inflammatory Antimicrobial	[45]
6	6.506	Syringic acid		0.45	Antioxidant Antimicrobial Anti-inflammatory	[46]
8	8.231	Rutin		0.42	Anti-inflammatory Immunomodulatory Antimicrobial Cancer treating activities	[47]
9	8.704	Ellagic acid		0.68	Anti-atherogenic Anti-inflammatory Neuroprotective Anticancer	[39]
11	9.741	Vanillin		0.22	Anticarcinogenic anti-inflammatory Antiviral Antioxidant	[53]
12	10.046	Ferulic acid		0.29	Anti-inflammatory Antioxidant Antidiabetic Anticancer	[50]
14	12.132	Daidzein		0.31	Antioxidant Anti-inflammatory Anticancer	[51]
15	12.465	Quercetin		0.44	Antioxidant Anticarcinogenic Anti-inflammatory Anti-diabetic Antimicrobial	[54]
16	13.604	Cinnamic acid		0.24	Antioxidant Antimicrobial Anticancer Neuroprotective anti-inflammatory Antidiabetic	[55]



**Fig. 8** HPLC chromatogram of ethanol extract from guava seeds

to [37]. On the other hand, guava seeds had different phenolic compounds but with lower concentration than mango seeds which might be due to type of solvents or extraction method which can be modified in further studies. The presence of high amount of gallic acid, naringenin, and ellagic acid refers to its antioxidant and anticancer potential activity as stated by [37, 38], and [39]. The phytochemical constituents of mango extracts were higher than guava, thus mango had high antioxidant activity as stated before in antioxidant tests as there was a direct correlation between total antioxidant activity and total phenolic content (gallic acid, hydroxybenzoic acid, chlorogenic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, and sinapic acid) for the phytochemical extracts of several fruits as fruits with higher total phenolic content also had higher overall antioxidant activity, suggesting that phenolics may be a significant factor in the overall antioxidant activities of fruits [40].

## 4 Conclusion

From the obtained results, it could be concluded that mango ethanolic extract contains a high amount of phenolic compounds that could be used as antioxidants which was approved by the HPLC results showing that it contained high concentration of gallic acid, naringenin, ellagic acid, and

ferulic acid as they have anticancer and antioxidant activities. The obtained results of ethanolic mango and fractions (nos. 6 and 8) achieved high antioxidant activity compared with other crude extracts and ascorbic acid as an antioxidant standard which is present with high concentration in mango ethanolic extract (1274.91 ppm). Additionally, ethyl acetate extract of guava showed strong cytotoxic effect on HepG2 cells in a dose-dependent manner in all tested concentrations with  $IC_{50}$  75.5 ppm when compared with mango extracts. Also the obtained results revealed strong correlation between antioxidant and phenolic compounds content in fruit seeds ( $r=0.87$ ) and moderate correlation between the antioxidant and anticancer activities with  $r=0.62$ .

**Author contribution** Conceived and designed the experiments: EAS, AMA, AAD. Performed the experiments: MM and EMM. Collection of data: MM, EMM, AMA EAS. Analyzed the data: MM, EMM, AMA EAS. Contributed reagents/materials/analysis tools: MM, EMM, AMA EAS. Wrote the paper: MM, EMM, AMA EAS. Revising of the manuscript: MM, EMM, AMA EAS.

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**Availability of data and materials** All data is available under request.

## Declarations

**Ethics approval and consent to participate** Not applicable in this section.

**Consent for publication** All authors read and approved the final manuscript.

**Competing interests** The authors declare no competing interests.

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