Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

The Remarkable Bioactivities of Desert Wild Herbs Stipagrostis plumosa, Artemisia monosperma, and Thymelaea hirsuta

Safwa M. Elderwy
October University of Modern Sciences and Arts
Mostafa Gouda
mostafa-gouda@zju.edu.cn

Zhejiang University
Ahmed M. Aboul-Enein
Cairo University
Nada R. Abdelgawad
October University of Modern Sciences and Arts
Abdelrhman E. Elsayed
October University of Modern Sciences and Arts
Mohamed Khalaf
October University of Modern Sciences and Arts
Randa S. Hasan
Agricultural Research Center
Emad A. Shalaby
Cairo University

Research Article

Keywords: Wild herbs phytochemistry, anticancer agents, antioxidant activity, HPLC separation, MTT cytotoxicity, antiviral assay

Posted Date: October 12th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10385960/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

The Remarkable Bioactivities of Desert Wild Herbs *Stipagrostis plumosa*, *Artemisia monosperma,* and *Thymelaea hirsuta*

Running title: Bioactivities of Three Wild Plants Grown in Desert

Safwa M. Elderwy[1], Mostafa M. Gouda[2,3,4,*], Ahmed M. Aboul-Enein[2], Nada R. Abdelgawad[1], Abdelrhman E. Elsayed[1], Mohamed Khalaf[1,] Randa S. Hasan[5] and Emad A. Shalaby[2,*]

[1] Faculty of Biotechnology, October University for Modern Science and Arts (MSA), Giza, Egypt.

[2] Department of Biochemistry, Faculty of Agriculture, Cairo University, Giza, 12613, Egypt.

[3] College of Biosystems Engineering and Food Science, Zhejiang University, 866 Yuhangtang Road, Hangzhou, 310058, China.

[4] Department of Nutrition & Food Science, National Research Centre, Dokki, 12622, Giza, Egypt.

[5] Regional Center for Food and Feed (RCFF), Agricultural Research Center (ARC) P. Box 588, Orman, Giza, Egypt.

* Corresponding authors.

Authors' email addresses: safwa.mohamed@msa.edu.eg (**S.M. Elderwy**), Mostafa-gouda@zju.edu.cn (**M. Gouda**), aboul.enein1@gmail.com (**A.M. Aboul-Enein**), nadarezk2020@gmail.com (**N.R. Abdelgawad**), abdelrhman.essam@msa.edu.eg (**A.E. Elsayed**), khalafmoh12@msa.edu.eg (**M. Khalaf**), randasaad@arc.sci.eg (**R.S. Hasan**), and emad.ahmed@agri.cu.edu.eg (**E. Shalaby**).

Abstract

Screening the bioactive potential of wild desert plants requires integration of analytical, phytochemical, and biological methods. This study evaluated the antioxidant, anticancer, and antiviral properties of three Egyptian wild plants (*Stipagrostis plumosa*, *Artemisia monosperma*, and *Thymelaea hirsuta*) through successive ethanolic extracts, fractionation, and chemical profiling. Antioxidant activities were assessed by five assays (DPPH, ABTS, methylene blue, $KMnO_4$, and hydrogen peroxide scavenging), while anticancer effects were tested against HepG2 cells and antiviral activity against human coronavirus 229E. Fractionation of the ethanolic extract of *A. monosperma* yielded bioactive fractions with strong radical-scavenging properties. HPLC analysis revealed high levels of quercetin (2543 µg/mL), naringenin (144 µg/mL), and chlorogenic acid (141 µg/mL), compounds closely associated with the observed activities. Notably, *S. plumosa* exhibited the strongest antioxidant capacity (91.8% ABTS scavenging), while *A. monosperma* and *T. hirsuta* showed significant anticancer effects (IC_{50} = 741.9 and 590.9 ppm, respectively) and moderate antiviral activity (IC_{50} = 35.1 µg/mL). In conclusion, the results demonstrate the richness of *A. monosperma* as a promising source of natural antioxidants (especially quercetin and chlorogenic), anticancer, and antiviral agents.

Keywords: Wild herbs phytochemistry; anticancer agents; antioxidant activity; HPLC separation; MTT cytotoxicity; antiviral assay.

1. Introduction

In the search for novel therapeutic agents, nature remains an unparalleled resource, providing an array of bioactive compounds with the potential to address pressing global health challenges. With increasing resistance to synthetic drugs and the rise of chronic diseases such as cancer, cardiovascular diseases, and viral infections, natural plant-based compounds have gained significant attention for their therapeutic properties. Among the many plants with promising medicinal value, those growing in harsh environments, such as deserts, are particularly interesting due to their remarkable resilience and ability to synthesize potent bioactive compounds [1-3]. These natural extracts have high potential in regulating the world's deadliest diseases like hepatocellular carcinoma (HCC), which is ranked as the fourth most prevalent cancer-related cause of death in the world [4]. Additionally, among the risk factors significantly affecting the administration of natural extracts are viral infections, which have been found to result in oxidative stress, primarily due to virally induced disruptions in the cell's antioxidant defense mechanisms [5,6].

In addition, oxidative stress is a key contributor to the pathogenesis of various diseases, resulting from external factors such as pollution and radiation, which can lead to significant cellular damage, including DNA mutations, lipid peroxidation, and mitochondrial dysfunction. This damage underpins many chronic diseases, including liver cancer (Hepatocellular carcinoma, HCC) and viral infections. As antioxidants play a central role in neutralizing ROS, they are considered crucial in reducing the risk of such diseases. However, synthetic antioxidants often pose health risks, making plant-based antioxidants a safer and more sustainable alternative. Several recent studies have highlighted the role of plant-based phenolic compounds and flavonoids in combating oxidative stress and improving human health. For instance, a study by Pandey and Rizvi [7] found that polyphenolic compounds from plant herbs exhibit strong antioxidant activity and offer protective effects against oxidative stress-induced

diseases, including cancer. Similarly, a study by Lv, et al. [8] demonstrated the antioxidant potential of flavonoids from Kiwi leaves, contributing to reduced oxidative stress and cancer prevention.

In this context, plant-derived compounds have shown promise in reducing tumor burden and enhancing the efficacy of conventional cancer therapies [9]. *Artemisia monosperma*, for example, contains several flavonoids, including quercetin and naringenin, which have been extensively studied for their anticancer properties. Quercetin, as reported by Liu, et al. [10], induces apoptosis in hepatocellular carcinoma (HepG2) cells by disrupting mitochondrial integrity. In contrast, naringenin has been shown to inhibit tumor cell proliferation by modulating various signaling pathways involved in cell survival and apoptosis [11]. These findings align with our study, where *A. monosperma* exhibited significant anticancer activity against HepG2 cells, with half-maximal inhibitory concentrations (IC_{50}) values of 741.92 ± 102.7 ppm, similar to other plant extracts known for their potent anticancer properties.

The antiviral properties of plant-derived compounds have garnered considerable interest in recent years, particularly as the world continues to battle emerging viral diseases, including COVID-19. Recent research has shown that flavonoids and phenolic compounds from plants can inhibit viral replication by interfering with the virus-host cell interaction, inhibiting viral enzymes, or modulating the immune response. For example, *Echinacea purpurea* extract, known for its antiviral properties, was found by Kolev, et al. [12] to inhibit the replication of the herpes simplex virus (HSV) through direct interactions with the viral envelope. Similarly, quercetin has been reported to exhibit antiviral effects against the SARS-CoV-2 virus by inhibiting viral entry and replication. Our study corroborates these findings, with *A. monosperma* exhibiting promising antiviral activity against 229E, which may be attributed to its high concentrations of antiviral flavonoids, such as quercetin and naringenin.

Stipagrostis plumosa (L.) Munro ex T. Anderson, *Artemisia monosperma* Delile, and *Thymelaea hirsuta* (L.) Endl represents three promising wild plants native to Egypt's desert regions. These plants are rich in secondary metabolites, such as phenolic acids, flavonoids, alkaloids, and terpenoids, which have been associated with a range of bioactivities, including antioxidant, anticancer, and antimicrobial effects. While *A. monosperma* has been studied for its high concentrations of quercetin and naringenin, *T. hirsuta* is noted for its chlorogenic acid content, which has demonstrated antioxidant and anticancer effects. Recent studies have shown that chlorogenic acid possesses anti-inflammatory and anticancer properties by inhibiting the growth of tumor cells and promoting apoptosis. In our study, *T. hirsuta* exhibited significant antioxidant activity and displayed moderate anticancer effects, supporting the growing body of evidence for its medicinal properties.

The comparative analysis of these plants with other recent studies emphasizes their significant therapeutic potential. For instance, *S. plumosa*, with its high phenolic content, exhibited the highest antioxidant activity, confirming its bioactivities. This comparative approach highlights the significance of these wild Egyptian plants in the global quest for new, natural, and effective therapeutic agents [13]. Many of these species' biological functions remain unknown. Despite recent evidence of the nutritional and health benefits of these plants, many countries underutilize their components in food and therapy due to limited research, data, and processing techniques [14].

For instance, *Stipagrostis plumosa Munro ex T. Anderson* is a type of perennial grass that is drought-tolerant and is frequently seen in desert areas. It belongs to the *Gramineae* or *Poaceae* families. Various bioactive compounds include alkaloids, hydroxamic acids, phenolic acids, terpenoids, flavonoids, fatty acids, and steroids. Pharmacologically speaking, Poaceae plants have a wide range of bioactivities, such as cytotoxic, analgesic, anti-inflammatory,

antipyretic, hepatic-protective, and antioxidant properties [15]. *Monospermous Artemisia Delile* is a genus of tiny shrubs and herbs of the Asteraceae family [16]. One of the most common and poorly understood species found along Egypt's Mediterranean shore is *Artemisia monosperma Delile*.

Many *Artemisia* species generate oil that is useful in medicine. The anticancer, antimalarial, antispasmodic, antihypertensive, and antioxidant properties of *A. monosperma* account for its therapeutic significance [17]. Because *A. monosperma* contains a large number of secondary metabolites, including oils, terpenoids, coumarins, flavonoids, alkaloids, and acetylenic chemicals, it displays a broad spectrum of biological activity [16]. In addition, members of the *Thymelaeaceae* family are frequently used in folk medicine to cure various ailments, such as leukemia, tumors, gout, inflammation, and infection. *T. hirsuta* is a poisonous plant with some medical uses. *T. hirsuta* ethanolic extracts have a broad range of antibacterial and antifungal properties in addition to vigorous antioxidant activity, and they can significantly suppress the growth of human adenocarcinoma cells [18].

This study evaluated the antioxidant, anticancer, and antiviral activities of successive ethanolic extracts of *S. plumosa*, *A. monosperma*, and *T. hirsuta*, along with their phytochemical profiles. Unlike previous research, it included multiple antioxidant assays, cytotoxicity tests against HepG2 cells, antiviral tests against human coronavirus 229E, and HPLC profiling of phenolics and flavonoids. Where fractionation of *A. monosperma* identified several active sub-fractions. The study's originality lies in linking the bioactivities of the three compared desert plants to their compounds, such as quercetin, naringenin, and chlorogenic acid, revealing structure–activity insights. Therefore, it highlights their potential in functional foods, nutraceuticals, and pharmaceuticals.

2. Materials and methods

2.1 Chemicals and reagents

Pure hexane, ethyl acetate, ethanol, and methanol were purchased from E. Merck Co. (Darmstadt, Germany). Sulfarhodamine, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2, 2-azino-bis (ethylbenzthiazoline-6-sulfonic acid (ABTS+), gallic acid, butylated hydroxyl toluene (BHT), potassium permanganate ($KMnO_4$), methylene blue, ascorbic acid, DCPIP solution, and hydrogen peroxide were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ferric chloride was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Copper sulphate, ammonia, lead acetate, sodium carbonate, aluminum chloride ($AlCl_3$), potassium acetate, and hydrochloric acid (HCl) were purchased from VladaChem GmbH (Malsch, Germany). Sodium hydroxide was purchased from Hua-Chem (Villingen-Schwenningen, Germany). GAA, Wigner reagent, and Benedict reagent were purchased from Sigma (Washington, USA). DMEM media were purchased from Lonza (Basel, Switzerland). The MTT (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyltetrazolium bromide) was obtained from Merck KGaA (Darmstadt, Germany). Vero-E6 cells and 229E coronavirus cells were purchased from Sigma, Washington, USA. A microplate ELISA reader was purchased from (FLU Ostar OPTIMA, BMG LABTECH GmbH, Ortenberg, Germany). The Anthos Zenyth 200rt plate reader was purchased from Anthos Labtech Instruments, Heerhugowaard, Netherlands.

2.2 Collection of wild plant materials

This is shown in **Figure** 1 for the experimental design of the overall flowchart. The leaves and stem of *Stipagrostis plumosa (L.) Munro ex T. Anderson*, *Artemisia monosperma Delile* and *Thymelaea hirsuta (L.) Endl* were collected from Al Alameen City, Northern coast of Egypt, in August 2022. The samples were then prepared for the downstream experiments.

The formal identification of all plant species was conducted by Prof. Dr. Rim Hamdy, a Botanical expert, Department of Botanical Science at Cairo University's Faculty of Agriculture. Voucher specimens of *Stipagrostis plumosa* (Voucher No. SP2022), *Artemisia monosperma* (Voucher No. AM2022), and *Thymelaea hirsuta* (Voucher No. TH2022) were deposited in the Herbarium of the Department of Botany, Faculty of Science, Cairo University, Egypt, and are publicly available upon request. The authors confirm that all necessary permissions for the collection of wild plant samples were obtained from the relevant authorities in Egypt before field collection.

2.2.1 Compliance Statement

The collection and use of plant materials in this study complied with all relevant institutional, national, and international guidelines and legislation. No endangered or protected species were involved.

2.3 Plant extraction

The collected wild plants were air-dried and ground to a fine powder. The dried powders (7.9 g of *S. plumosa*, 13.0 g of *A. monosperma*, and 13.9 g of *T. hirsuta*) were subjected to successive extraction with 70% ethanol, repeated three times according to Pvman [19]. The organic solvent extracts were concentrated in a water bath at 40–50 °C to remove solvent. The percentage yields of the crude ethanolic extracts were calculated based on the initial dry plant weight: *S. plumosa* (12.5%), *A. monosperma* (15.3%), and *T. hirsuta* (14.7%). These yields reflect the relative abundance of extractable phytochemicals present in each plant material [2].

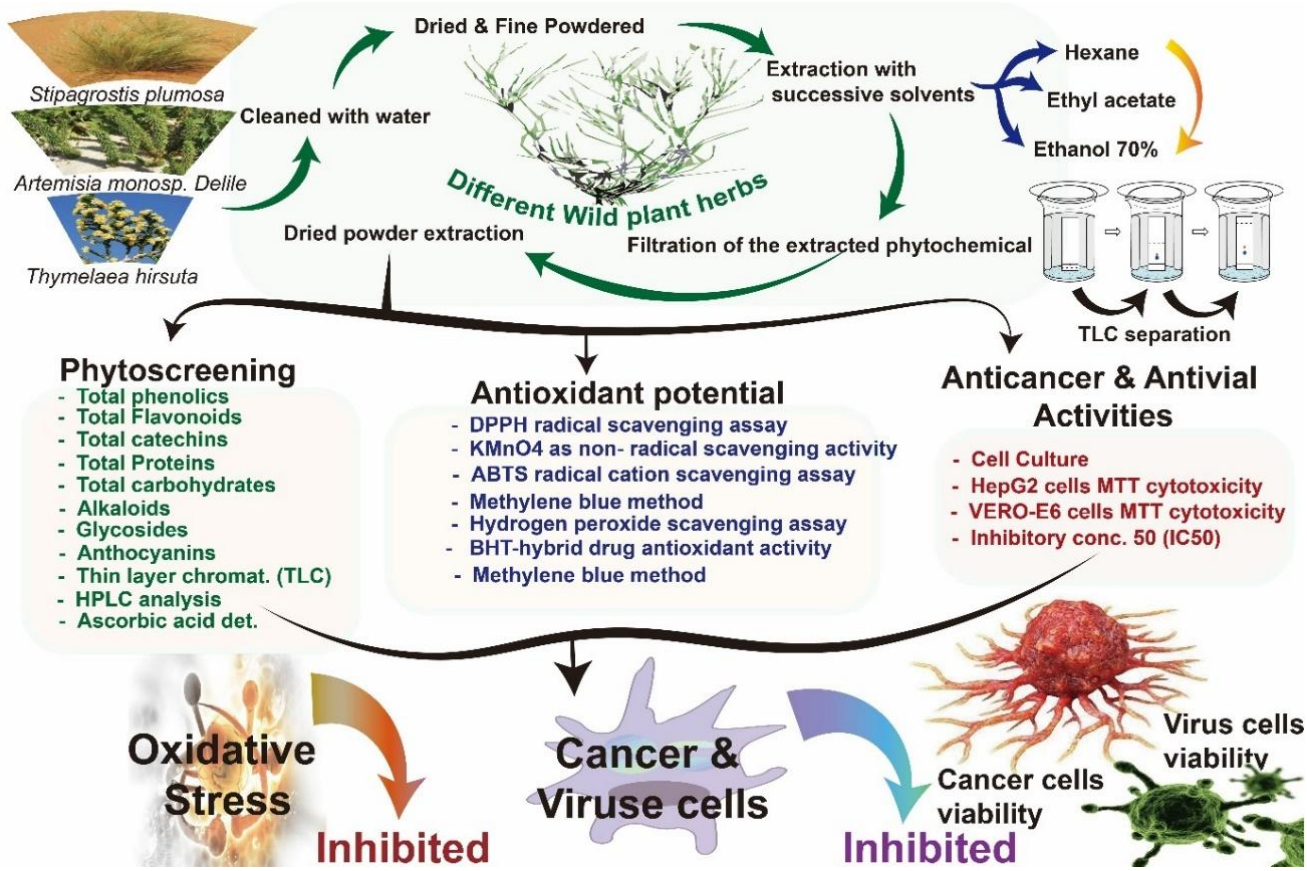


Figure 1 presents the overall flowchart of the experimental design.

2.4 Qualitative phytochemical screening

Qualitative phytochemical analysis of *S. plumosa (L.) Munro ex T. Anderson, A. monosperma* and *Thymelaea hirsute (L.) Endl* extracts were done by the following method:

2.4.1 Ferric chloride test for phenolic compounds

A few drops of a ferric chloride solution (5%) were mixed with aqueous extract solutions. The resultant color is a dark green that is bluish-black [20].

2.4.2 Test for carbohydrates

The extracts were heated immediately after applying the Benedict Reagent to detect carbohydrates. A positive result showed a reddish-orange precipitate [21]. The test involved mixing 1 mL of extract with 1 mL of Benedict's reagent, then heating in boiling water for 5-

10 min. A color change from blue to red-orange indicated reducing sugars, as these sugars reduce copper(II) ions to copper(I) ions. The darker the color, the more reducing sugar present.

2.4.3 Total Protein analysis

The total protein was assessed using Shaikh and Patil [20]. In brief, 1 mL of copper sulfate was mixed with five drops of the extract solutions, then stood for 10 min at room temperature. Proteins caused a violet color by binding copper ions to peptide bonds, forming a colored complex. The violet color's intensity was measured at 540 nm spectrophotometrically. Protein levels were determined by comparing absorbance to a BSA standard curve.

2.4.4 Amino acids analysis

Amino acids were detected in the plant extracts using the ninhydrin reaction, as outlined by Shaikh and Patil [20]. To 1 mL of the plant extract, 3 drops of 1% sodium hydroxide (NaOH) were added, and the mixture was heated for 3-5 min in a boiling water bath. After cooling, 1-2 drops of an indicator (e.g., phenolphthalein) were added. The appearance of a pink color indicated the presence of amino acids, which react with the alkaline solution to form a colored complex. The intensity of the pink coloration was proportional to the concentration of amino acids in the sample, where a positive outcome resulted in a pink color [20].

2.4.5 Test for flavonoids

The presence of flavonoids in the plant extracts was tested using the lead acetate method, as described by Gouda, et al. [22]. To 1 mL of the plant extract, 5 drops of a 10% lead acetate solution were added. A positive result was indicated by forming a yellow precipitate, which occurs when lead ions (Pb^{2+}) react with the hydroxyl and carbonyl groups in the flavonoid structure. A yellow precipitate was a qualitative indication of flavonoid compounds

in the extract. Five drops of lead acetate were added to each of the five extracts for the test, until a yellow precipitate was observed, indicating a positive result.

2.4.6 Test for anthocyanins

The presence of anthocyanins, water-soluble pigments responsible for red, purple, and blue colors in plants, was tested using hydrochloric acid (HCl) and ammonia solution, as outlined by Gouda, et al. [23]. To 1 mL of the plant extract, 10 drops of 2N HCl and 1 mL of ammonia solution were added. A positive result was indicated by a color change to violet or blue, which occurs when anthocyanins react with the acidic and alkaline solutions. The color change is due to pH-dependent structural changes in anthocyanin molecules, which confer distinct colors under varying pH conditions. A purple or blue tint in the solution confirmed the presence of anthocyanins in the extract.

2.4.7 Analysis of Coumarins, glycosides and alkaloids

Ten drops of sodium hydroxide were mixed with five drops of the extract. Yellow indicates a positive result [20]. For glycosides, 10 drops of GAA and 1 mL of ferric chloride were added; a brown ring at the interface indicates a positive reaction, where glycosides break, releasing sugars that react with ferric ions. Alkaloids were tested using Wagner's reagent: 1 mL of extract with 5 drops of Wagner's reagent produces a reddish-brown precipitate, indicating alkaloids, which react with iodine to form a colored complex. This test relies on alkaloids precipitating upon reaction with iodine [20].

2.5 Thin-layer chromatography (TLC)

The plant extracts were analyzed using thin-layer chromatography (TLC) to separate the active compounds. TLC was performed on pre-coated silica gel plates (TLC F254). Benzene: acetone (9:1 v/v) was used as the mobile phase, and the separated compounds were

visualized after exposure to the DPPH reagent. The presence of antioxidant compounds was confirmed by the formation of yellow or white bands on a purple background [24].

2.6 Determination of phenolic compounds of the separated compounds by TLC

The procedure outlined by Singleton [25] was used to determine phenolic compounds. In brief, 750 µL of 10% Folin-Ciocalteu reagent was mixed with 100 µL of each extract. After three minutes, 750 µL of saturated sodium carbonate (6%) was added. The mixture was kept in the dark for 725 hours, then the absorbance at 725 nm was measured. Gallic acid served as the standard for the calibration curve, and total phenolic content was expressed as mg GAE per gram of extract, calculated from the gallic acid standard curve (5- 100 µg/mL) using the following formula:

$$\text{Phenolic content (mg GAE/g extract)} = (A \times V)/(C \times m) \quad (1)$$

Where A is the absorbance of the sample, V is the final volume (mL), C is the concentration of the gallic acid standard (µg/mL), and m is the mass of the extract (g).

2.7 Determination of Total Flavonoids

The method by Zhishen, et al. [26] was used to identify the flavonoid compounds. In brief, 150 µL of $AlCl_3$ (10%, w/v) was combined with 125 µL of each extract. After 5 min, 750 µL of 1 M NaOH was added. For 15 min, the reaction was incubated in the dark. After thorough mixing, absorbance was measured at 510 nm against a blank. The calibration curve used quercetin as the standard, prepared by diluting stock solutions to 0-100 µg/mL. Flavonoid content was expressed as mg quercetin equivalents (QE) per gram of extract, using the same equation as Phenolic content (Equ.1).

2.8 Determination of total catechins

With simple modifications, the vanillin assay reported by Hayat, et al. [27], Belyagoubi-Benhammou, et al. [28] was used to measure total catechin tannin. In brief, 1500 µL of a vanillin/methanol (4%) solution was added to 50 µL of this extract and thoroughly mixed. Next, 750 µL of concentrated HCl was added, and the mixture was left to react for one h at room temperature. A blank was used to measure the absorbance at 550 nm. Using the catechin calibration curve as a reference, the total concentration of condensed tannins was reported in micrograms of catechin equivalents per milligram dry matter.

2.9 Ascorbic acid determination

Ascorbic acid content was determined using the DCPIP (2,6-dichlorophenolindophenol) titration method. The DCPIP solution was freshly prepared by dissolving 200 mg of DCPIP, 106 mg of K_2HPO_4, and 90 mg of NaH_2PO_4 in 1000 mL of double-distilled water. The solution was calibrated against a standard ascorbic acid (AA) solution before use to ensure accuracy and stability (valid for up to 30 days under refrigerated storage). DCPIP serves as a redox dye, changing color from blue to pink upon reduction by ascorbic acid, which enables visual detection of the titration endpoint. For each sample, the plant extract solution was titrated with the standardized DCPIP solution, and the ascorbic acid concentration was calculated based on the volume of DCPIP consumed, using the ascorbic acid standard calibration curve [29].

2.10 Antioxidant activity by DPPH

For the DPPH measurement, the modified method of Gouda, et al. [23] was used to conduct the test. In brief, 1 mL of DPPH radical solution (1X) was mixed with 1 mL of sample extract and then left to stand for 5 min. The positive results were pinkish to colorless, which were measured by spectrophotometer (Thermo-Scientific, IL, USA). The scavenging effects of extracts from *S. plumosa (L.) Munro ex T. Anderson, A. monosperma,* and *Thymelaea irsute*

(L.) Endl were ascertained. A test tube with 1 mL of sample extract was filled with 2 mL of 0.16 mM DPPH solution, vortexed for a minute, then left in the dark at room temperature for thirty minutes. Absorbance at 517 nm was measured for each sample and BHT, a standard. The % scavenging activity was calculated as: % Antioxidant activity = (Control - Sample) x 100 / Control, where control is DPPH solution (0.16 mM).

2.11 potassium permanganate ($KMnO_4$) as non- radical scavenging activity

The method of Gaber, et al. [30] was used to assess the scavenging effects of the successive extracts. In brief, 1 mL of $KMnO_4$ solution was added to one mL of the sample extract for the test, and it was then left in the dark for thirty min. Next, take a reading at 514 nm. Blank (zero) was exclusively prepared by distilled water. $KMnO_4$ reagent, which measured at 514 nm, served as the control. To make the standard (ascorbic acid), mix 1 mL of ascorbic acid with one mL of $KMnO_4$ reagent. Incubate in the dark for thirty min, and then measure at 514 nm. The scavenging activity percentage (%) was computed as follows: % Antioxidant activity = (Control-Sample x 100) / Control Where: control $KMnO_4$ solution.

2.12 ABTS radical cation scavenging assay

The experiment was based on how well various compounds scavenged the radical cation of 2, 2'-azino-bis ethylbenzthiazoline-6-sulfonic acid ($ABTS^+$) in relation to a standard (BHT). In order to prepare the radical cation, 2.45 mM potassium persulfate (1/1, v/v) was combined with 7 mM ABTS stock solution. The combination was then left for 4–16 h, or until the reaction was finished and the absorbance remained stable. According to Lv, et al. [8], the ($ABTS^+$) solution was diluted with ethanol until it gave an absorbance of 0.700 ± 0.05 at 734 nm for measurements. To conduct tests, 0.5 mL of each extract was added to 1 mL of ABTS reagent, shaken for a minute, and then measured at 734 nm. A blank was prepared with ethanol. For ascorbic acid, 0.5 mL was mixed with 1 mL of ABTS, measured at 734 nm, and kept in

darkness for 30 min. The control was ABTS reagent. The percentage of scavenging activity was calculated as: % Antioxidant activity = (Control - Sample) x 100 / Control, where control is ABTS reagent.

2.13 Methylene blue method

The following criteria were used to assess the crude extract's scavenging potency: Test tubes holding 1 mL of extract sample were filled with 1.0 mL of 0.002 M methylene blue solution (in water), left in the dark for 30 min, and then measured at 663 nm. Distilled water alone was used to prepare the blank (zero), and the methylene blue reagent was used as the control. 1 mL of ascorbic acid was combined with 1 mL of methylene blue reagent to create the standard, which was then incubated as previously[30]. The scavenging activity percentage (%) was computed as follows:

% Antioxidant activity = (Control-Sample x 100) / Control (2)

2.14 Hydrogen peroxide scavenging assay

The assay was determined by Dandare [31] . Aliquot of 0.5 mL of extracts (50 µg/mL) was transferred into the nine tubes of three extracts followed by the addition of one m L of H_2O_2 solution (2 mM), vortexes for ten min and measured at 320 nm against Blank. Ascorbic acid was used as the positive control. The ability of the extract to scavenge the H_2O_2 was calculated using the following equation:

H_2O_2 scavenging activity percentage = $[(A_0-A_1)/A_0] \times 100$ (3)

, where: A_0 = Absorbance of control, A_1 = Absorbance of sample.

2.15 Fractionation of ethanolic extract as a promising extract

Hexane was used as the solvent while 150g of silica gel (60–120 mesh) was placed into the 40 cm long by 2.5 cm wide chromatographic column. After being finely mixed with silica

337 gel powder, five g of *Artemisia monosperma Delile's* ethanol crude extract—a promising crude
338 extract—were added to the top of the packed column. The polarity of the column was then
339 increased by 30% between each mobile phase mixture[32]. after it had been progressively eluted
340 with 100% hexane and ethyl acetate and ethanol solvent. As a result, eight fractions were
341 produced in **Table** 1.

Table 1. Fractions collected from successive extracts of *Artemisia monosperma Delile,* using column chromatography.

Solvent/Sample no.	**Fractions No.**							
	1	2	3	4	5	6	7	8
Hexane	100	70	40	10	0	0	0	0
Ethyl acetate	0	30	60	90	100	70	40	0
Ethanol	0	0	0	0	0	30	60	100

342

343 ## *2.16 Anticancer test*

344 ### 2.16.1 Cells

345 The study utilized human epithelial hepatocellular carcinoma cells (HepG2) obtained
346 from the Faculty of Biotechnology, October University for Modern Sciences and Arts (MSA),
347 Giza, Egypt. These cells were originally purchased from the American Type Culture Collection
348 (ATCC, Manassas, VA, USA; catalog no. HB-8065). The cells were subsequently activated
349 and cultivated according to the method of Gouda, et al. [33].

350 ### 2.16.2 Cell Culture

351 The study employed HepG2, a human epithelial-like hepatocellular cancer. The cells were
352 regularly grown in DMEM supplemented with 10% fetal bovine serum (FBS), 2 mM L-
353 glutamine, 100 units/mL of streptomycin sulphate, 250 ng/mL of amphotericin B, and 100
354 units/mL of penicillin G sodium. Cells were kept in humidified air with 5% CO_2 at a sub-

confluence temperature of 37ºC. After trypsin/EDTA treatment at 37°C, monolayer cells were extracted for sub-culturing. Once confluence had reached 75%, cells were used.

2.16.3 MTT Cytotoxicity

Based on the ability of living cells' active mitochondrial dehydrogenase enzyme to cleave the yellow MTT's tetrazolium rings and generate dark blue insoluble formazan crystals, the cytotoxicity of the tested materials was evaluated. When the crystals dissolve, a dark blue color equal to the number of living cells is released. A flat bottom 96-well microplate containing 1×10^4 cells /well was seeded with a serum-free medium. The cells were then treated with 20 µL of various doses of the tested substances for 24 h at 37º C in a humidified 5% CO_2 atmosphere. Following incubation, the media were taken out, and 40 µL of MTT solution per well were added. Incubation was then continued for four h. Following the solubilization of MTT crystals with 180 µL of acidified isopropanol per well and plate shacking at room temperature, the absorbance at 570 nm was measured photometrically using a microplate ELISA reader. For every concentration, three repetitions were made, and the average was determined [34].

2.17 In vitro bioassay of cytotoxicity, antiviral activity and mode of actions

2.17.1 MTT cytotoxicity assay

Stock solutions of the test compounds were produced in 10% DMSO in ddH2O and then diluted to the working solutions with DMEM in order to determine the 50% cytotoxic concentration (CC_{50}). VERO-E6 cells were used to examine the extracts' cytotoxic activity using a slightly modified version of the 3-(4, 5-dimethylthiazol -2-yl)-2, 5-diphenyltetrazolium bromide (MTT) technique. In a nutshell, the cells were seeded at a density of 3×10^5 cells/mL in 96 well-plates with 100 µL of CO_2 in each well, and they were incubated for 24 h at 37 °C. Cells were treated in triplicate with varying doses of the investigated substances after a 24-h

period. The supernatant was disposed of 24 h later, and the cell monolayers were rinsed three times with sterile 1x phosphate buffer saline (PBS). After that, 20 µL of the 5 mg/mL stock solution of MTT solution was added to each well, and the mixture was incubated for four h at 37 °C before medium aspiration. A solution of 200 µL acidified isopropanol (0.04 M HCl in absolute isopropanol = 0.073 mL HCL in 50 mL isopropanol) was used to dissolve the formazan crystals that had formed in each well. Using a multi-well plate reader, the absorbance of formazan solutions was measured at λmax 540 nm, with 620 nm serving as a reference wavelength. Using GraphPad Prism 5 software, the percentage of cytotoxicity relative to the untreated cells was calculated by charting the percentages of cell viability and viral inhibition versus concentration for each.

2.17.2 Inhibitory concentration 50% (IC_{50}) determination

The IC_{50} values of the studied compound were determined. In summary, 2.4×10^4 Vero-E6 cells were seeded into each well of 96-well tissue culture plates, and the plates were incubated for the entire night at 37°C in a humidified environment with 5% CO_2. After one 1x PBS wash, the cell monolayers were exposed to 1 h of room temperature (RT) 2 29E virus (100 TCID50) adsorption. The investigated chemicals were added to 50 µL of DMEM containing different quantities, which was done on top of the cell monolayers. Following incubation at 37°C in 5% CO_2 incubator for 72 h, the cells were fixed with 100 µL of 4% paraformaldehyde for 20 min and stained with 0.1% crystal violet in distilled water for 15 min at RT. The optical density of the color was measured at 570 nm using an Anthos Zenyth 200rt plate reader after the crystal violet dye was dissolved using 100 µL of absolute methanol each well. According to Mostafa, et al. [35], the compound's half-life (IC_{50}) is the amount needed to decrease the viral-induced cytopathic effect (CPE) by 50% in comparison to the virus control.

2.18 High Performance Liquid Chromatography (HPLC) analysis

An Agilent 1260 series was used for the HPLC analysis. An Eclipse C18 column (4.6 mm x 250 mm i.d., 5 µm) was used for the separation. Water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) were combined to form the mobile phase, which was flowing at a rate of 0.9 mL/min. The following was the sequential linear gradient programming for the mobile phase: 8–12 min (60% A), 0–5 min (80% A), 12–15 min (82% A), 15–16 min (82% A), and 16–20 min (82% A). At 280 nm, the multi-wavelength detector was observed. For both the sample solutions *A. monosperma* and *T. hirsuta (L.) Endl*, the injection volume was 5 µL. The column was maintained at a constant temperature of 40 °C [36].

2.19 Statistical analysis

All experiments were performed in triplicate, and results are expressed as mean ± standard deviation (SD). Statistical analyses were conducted using one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) to determine significant differences among means. Differences were considered statistically significant at $p < 0.05$. Superscript letters are used to indicate significant differences between groups: values sharing the same letter are not significantly different, whereas values with different letters differ significantly at $p < 0.05$. Where relevant, exact p-values are also reported in Figure legends or Table captions for transparency. Statistical calculations and graphing were performed using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA).

3. Results and discussion

3.1 Qualitative phytochemical screening

There are numerous wild plants rich in secondary plant metabolites and important phytochemicals, known to possess antiviral, anticancer, and antioxidant properties.

Phytochemical analysis was performed on freshly made ethanolic extracts of *S. plumosa (L.) Munro ex T. Anderson*, *Artemisia monosperma Delile*, and *T. hirsuta (L.) Endl* to identify the presence of primary and secondary metabolites. **Table** 2 shows the screening of the plants under study's 70% ethanol extracts. The results of the investigation indicated that the extract from *A. monosperma* contained phenolic, sugar, fatty acid, flavonoids, and coumarins that exhibited antioxidant activity against $KMnO_4$. These were followed by extracts from *S. plumosa (L.) Munro ex T. Anderson* and *T. hirsuta (L.) Endl.* These outcomes were in line with those of Fatima et al. (2019), who discovered that the n-hexane extract of *S. plumosa (L.) Munro ex T. Anderson* contained antioxidants but no coumarins. The present results also showed the presence of fatty acids, and this can be due to a difference in the solvent. The results were also similar to those obtained by Costa (Costa et al., 2016), who found that the phytochemical analysis of *A. monosperma* revealed the presence of phenolic and flavonoids and the absence of glycosides in the dichloromethane extract. However, the results of this study showed the presence of other metabolites such as fatty acids, sugar, anthocyanins, coumarins, and antioxidants. Moreover, the present results also agreed with those of Amari [37], who found that the phytochemical analysis of *T. hirsuta (L.) Endl*. Contained coumarins in the ethanol extract.

Table 2. Phytochemical screening of Ethanol 70% extracts from *S. plumosa (L.) Munro ex T. Anderson, A. monosperma* and *T. hirsuta (L.) Endl.*

Phytochemical comp.	*S. plumosa (L.) Munro ex T. Anderson*	*Artemisia monosperma Delile*	*T. hirsuta (L.) Endl*
Phenolic	-	+	-
Sugars	-	+	-
Protein	-	-	-
Fatty acids	+	+	+
Flavonoids	-	+	-
Anthocyanins	-	-	-
Coumarins	-	+	+
Glycosides	-	-	-

Alkaloids	-	-	-
Antioxidant activity by $KMnO_4$	+	+	+
Antioxidant activity by DPPH Test	-	-	+

+: Found; -: Not found

3.2 Thin-layer chromatography (TLC)

Thin-layer chromatography (TLC) was used to separate and identify the compounds by successive extracts. Antioxidant potential compounds on TLC plates were identified in situ by using the DPPH reagent (**Figure** 2). The fractions producing yellowish or white bands on the purple background were considered strong antioxidants. The ethanol extract of *A. monosperma* showed a white or yellow band (antioxidant compounds) formed on exposure to DPPH. However, the ethanol extract of both *S. plumosa (L.) Munro ex T. Anderson* and *T. hirsuta (L.) Endl* contained small amounts of antioxidant compounds. Unfortunately, no studies were available to compare the three plants' antioxidant levels using TLC. However, a study conducted by Ismail, et al. [38] confirmed that *A. monosperma* has a significantly high level of antioxidant properties, and this was proven by the presence of a number of flavonoids.

3.3 Contents of total phenolic, Flavonoids, and Catechin

The obtained results showed that the extract of *T. hirsuta (L.) Endl* had the highest phenolic & flavonoid contents, while *A. monosperma* had the highest catechin, as shown in **Table** 3. A similar study was conducted by Abdelkader, et al. [39], but it reported that the total phenolic compounds of *S. plumosa* were higher than those obtained in our study. This may due to the use of different solvents (acetate acid). The data of the present study showed that the proportion of flavonoids and catechins was 25.17 ± 1.26% and 5.6 ± 0.28%, respectively, which were not detected in another study.

Moreover, the results of Zayyat, et al. [40] showed that the content of flavonoids in the aqueous extract from *A. monosperma* was 0.205%, which was similar to that obtained in the present study (214 ± 10.7%). The results obtained from the current study revealed that the total phenolic and flavonoid compounds in the ethanol extract from *T. hirsuta* were 1520 ± 76% and 223 ± 11.15%, respectively, with low catechin content (58 ± 2.9%). In this regard, Trigui, et al. [41] demonstrated that the total phenolic and flavonoid compounds in the hexane extract of *T. hirsuta* were 0.61 g/100g, but they could not detect flavonoids alone. Moreover, the results may differ when other solvents are used.

Table 3. Total phenolic, flavonoids, and catechin compounds (mg/100g) of the three different species ethanolic extracts.

Extract	*S. plumosa (L.) Munro ex T. Anderson*	*A. monosperma Delile*	*T. hirsuta (L.) Endl*
Phenolic	450.23 ± 22.5^{a}	1400 ± 70^{b}	1520 ± 76^{c}
Flavonoids	25.17 ± 1.26^{a}	214 ± 10.7^{b}	223 ± 11.15^{b}
Catechin	5.60 ± 0.28^{a}	75 ± 3.75^{c}	58 ± 2.9^{b}

Values are expressed as mean ± SD. Different alphabetical letters in the same row mean significantly different ($P < 0.05$).

3.4 Ascorbic acid content

The ascorbic acid contents in the three tested plant extracts are presented in **Table** 4. Ascorbic acid in the ethanolic extract of *A. monosperma* was 540 ± 27 mg/100g, which was higher than both of the other extracts (*S. plumosa* and *Thymelaea hirsute*) (60 ± 3 and 300 ± 15 mg/100g, respectively. According to Abdelkader, et al. [39], Younes, et al. [32] and Amari, et al.

[37] the three wild plants were rich in phenolic compounds and ascorbic acid and have high potency as antioxidants.

Table 4: Ascorbic acid content (mg/100g) of ethanol extracts of *S. plumosa (L.) Munro ex T. Anderson, A. monosperma* and *S. plumosa (L.) Munro ex T. Anderson.*

Extract	Ascorbic acid (mg/100g)
S. plumosa (L.) Munro ex T. Anderson	60.0 ± 3.0^{a}
A. monosperma Delile	540.0 ± 27.56^{c}
T. hirsuta (L.) Endl	300.0 ± 15.12^{b}

Values are expressed as mean ± SD. Different alphabetical letters mean significantly different ($P < 0.05$).

3.5 Antioxidant activity

Antioxidants protect living cells from the damage caused by unstable molecules known as free radicals. The benefits of this antioxidant are required for free radical conditions, function, and strength. Cancer can develop due to free radical damage [42]. An antioxidant molecule may prevent oxidation or delay the production of other molecules. The chemical reaction of electron transfer from one substance to another is called oxidation. Free radicals are created by oxidation reactions that cause chain reactions that damage cells. Antioxidants limit these chain reactions by removing free radical intermediates and reducing other oxidation reactions [42].

The antioxidant activity of the ethanol extracts of *S. plumosa (L.) Munro ex T. Anderson*, *A monosperma Delile* and *T. hirsuta (L.) Endl* was shown in **Table** 5 against DPPH, H_2O_2, ABTS, Methylene blue, and $KMnO_4$, compared with ascorbic acid as a standard. The ethanolic extract of *S. plumosa* has the highest antioxidant extract against DPPH (89.23 ± 4.46%), ABTS (91.77 ± 4.59%), and Methylene blue (59.08 ± 2.95%) followed by *A. monosperma* extract,

503 which gave results of antioxidant extract against DPPH 87.58 ± 4.38%, H_2O_2 17.01 ± 0.85%,
504 ABTS 79.81 ± 3.99% and $KMnO_4$ 74.9 ± 3.75%. The extract of *T. hirsuta* gave antioxidant
505 against ABTS as 84 ± 4.2% and $KMnO_4$ as 69.6 ± 3.48% compared with the antioxidant
506 activity of ascorbic acid as an antioxidant (**Table** 5).

507 The results of this research are consistent with those found by Fatima, et al. [43]. The
508 authors reported that the antioxidant capacity of the methanol extract of *S. plumosa* showed
509 increasing potential. The ethanolic extract of *S. plumosa* exhibited the most promising
510 antioxidant activity, followed by the antioxidant capacity determined by radical-scavenging
511 and hybrid reaction assays. In another study, *A. keiskeana* exhibited low DPPH radical
512 scavenging activity as 44.7% [44]. Generally, the antioxidant pattern of the extract is reflected in
513 its total phenolic/flavonoid content. The low level of antioxidants in A. keiskeana extract is
514 attributed to its lower phenolic/flavonoid content compared to *A. monosperma*. Accordingly,
515 Merghem, et al. [45] found that the antioxidant activity of the methanol extract of *T. hirsuta* was
516 33.83 %.

Table 5. Antioxidant activity (%) of *S. plumosa (L.) Munro ex T. Anderson*, *A. monosperma and Thymelaea hirsute (L.) Endl* extracts.

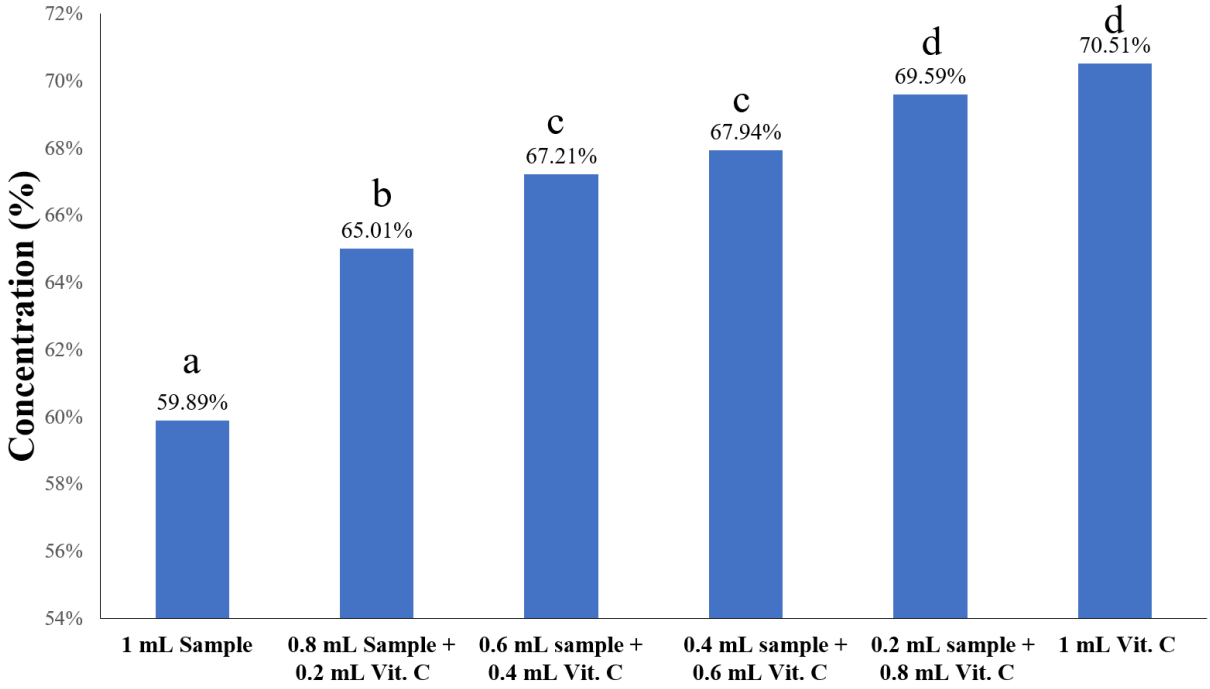
Parameter	***S. plumosa (L.) Munro ex T. Anderson***	***Artemisia monosperma Delile***	***Thymelaea hirsute (L.) Endl***	**Standard Ascorbic acid**
DPPH	89.23 ± 4.46^{b}	87.58 ± 4.38^{b}	62.69 ± 3.13^{a}	88.5 ± 4.43^{c}
H_2O_2	17.92 ± 0.90^{a}	17.01 ± 0.85^{a}	17.01 ± 0.85^{a}	50.12 ± 2.51^{b}
ABTS	91.77 ± 4.59^{b}	79.81 ± 3.99^{a}	84.01 ± 4.20^{a}	93.67 ± 4.68^{b}
Methylene blue	59.08 ± 2.95^{b}	60.37 ± 3.02^{b}	53.14 ± 2.66^{a}	72.51 ± 3.63^{c}
$KMnO_4$	**ND**	74.9 ± 3.75^{c}	69.6 ± 3.48^{b}	53.7 ± 2.69^{a}

517 Abbreviation: ND, not determined. Values are expressed as mean ± SD. Different alphabetical
518 letters at the same row mean significantly different ($P < 0.05$).

519

520 ## *3.6 Hybrid* reaction

521 The antioxidant activity of the extracts of *S. plumosa (L.) Munro ex T. Anderson* and *A.*
522 *monosperma Delile* using the hybrid reaction with Ascorbic acid (the natural standard anti-
523 oxidant) and DPPH was determined the results were given in (**Figure** 2). *S. plumosa* extract
524 showed high activity against 0.8 mL ascorbic acid by 69.59 ± 3.48%, and *A. monosperma*
525 showed antioxidant activity of 67.03 ± 3.35%. *S. plumosa* has analgesic, anti-inflammatory,
526 antioxidant, and cytotoxic activities against MCF-7 and HepG2cell lines [39].



527 **Figure 2.** Antioxidant activity (%) of the hybrid reaction between ethanolic extracts of *S.*
528 *plumosa (L.)* and *A. monosperma* with ascorbic acid (as standard antioxidant) against
529 DPPH at different concentrations..

530

531 The results of our research demonstrated that the promising ethanol S. plumosa extract
532 had high antioxidant activity in the Hybrid reaction at 2 mL of ethanolic S. plumosa extract
533 and showed values of 69.59 ± 3.48% compared to ascorbic acid alone (70.51 ± 3.53%). The

results are very convergent. In addition to the results of *Artemisia monosperma* extract had high antioxidant activity in Hybrid reaction at 2 ml of ethanolic *Artemisia monosperma* extract showed values of 67.03 ± 3.35% compared to ascorbic acid alone (69.59 ± 3.48%) are approximately the results are convergent According to Al-Soqeer [46], the *Artemisia monosperma* had high antioxidant activities.

3.7 Measurement of Antiradical activity.

The Antiradical activity of *A. monosperma* extracts using DPPH assay at 100 µg mL^{-1} was evaluated, and the obtained results indicated that the extract has high Antiradical capacity to scavenge DPPH (0.283 ± 0.01 AU) (**Figure** 3). The results also showed that the Antiradical activity increased by time of incubation. The highest value was 0.283± 0.01 AU and obtained after 40 sec. In this concern, Laouini[47] confirmed that *Artemisia monosperma* has high power antiradical activity.

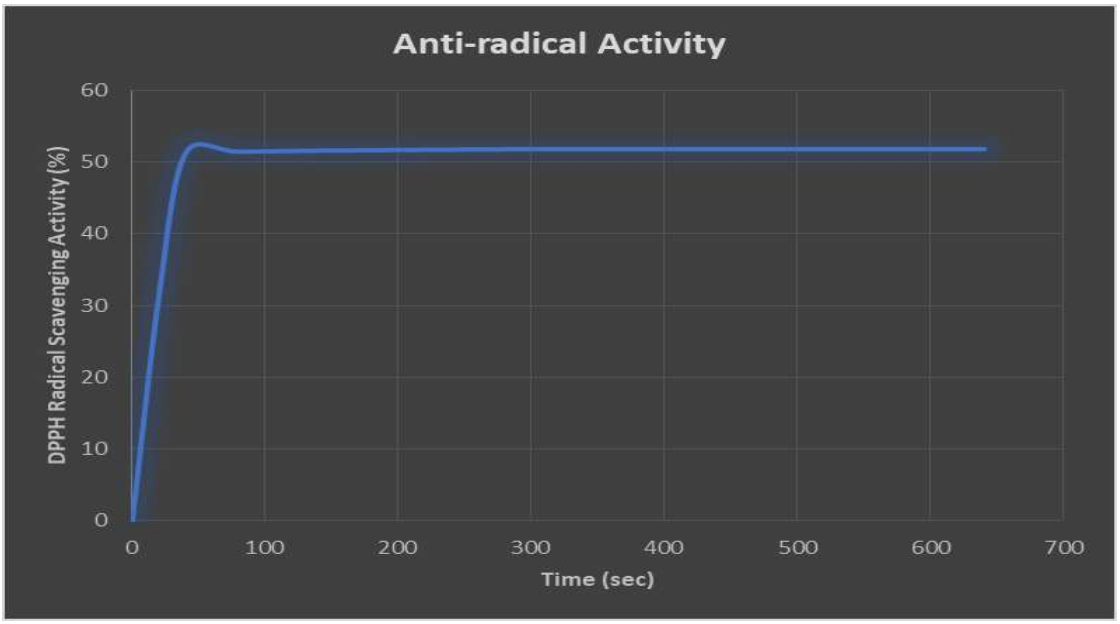


Figure 3. Antiradical activity (AU) of ethanol extracts of *A. monosperma* against DPPH at 00 µg mL^{-1}.

3.8 Fractionation of ethanolic wild plant extract as a promising extract

549 The obtained results showed that 8 fractions were separated and identified from the
550 promising ethanol extract of *Artemisia monosperma Delile* against two assays, ABTS (radical
551 assay) and Methylene Blue (nonradical assay). The antioxidant activity of each fraction was
552 evaluated at 100 µg/mL and given in **Table** 6, which showed that fraction Number (1) has high
553 biological activities as an antiradical against ABTS methods at 1 and 10 min by 90.29 ± 4.51%
554 and 89.36 ± 4.47%, respectively. Fraction Number (2) gave the same effect but with slightly
555 lower values (83.06 ± 4.15% and 88 ± 4.4% respectively) followed by fraction No. (4) (77.78
556 ± 3.89 and 83.14 ± 4.16 % respectively).

557 The obtained results in (**Table** 6) showed that fraction No.2 at 15 min and 30 min has
558 high antiradical activity against Methylene Blue by 62.95 ± 3.15% and 65.06 ± 3.25%
559 respectively, followed by fraction No.3 by 52.92 ± 2.65% and 68.99 ± 3.45% then fraction No.
560 1 by 59.90 ± 2.99% and 61.70 ± 3.09% respectively. Fraction No.1 (Hexane fraction) was the
561 best fraction as an antiradical, which may be due to the occurrence of compounds that have
562 high antiradical and, in turn, strong antioxidant activity. The extract contains Chlorogenic acid,
563 Naringenin, Daidzein, and Quercetin components. It also contains a high percentage of
564 phenolic, flavonoids, and catachin compounds as shown in the previous results of HPLC in this
565 research.

566

Table 6. Antioxidant activity (%) of ethanolic extract fractions of *A. monosperma Delile* against ABTS and methylene blue assays at 100 µg mL^{-1} with two incubation time.

Fractions no.	**Antioxidant activity %**			
	ABTS (Radical assay)		**Methylene blue** (non-radical assay)	
	1 min	10 min	15 min	30 min
1	90.29 ± 4.51^{f}	89.36 ± 4.47^{f}	59.90 ± 2.99^{de}	61.70 ± 3.09^{cd}
2	83.06 ± 4.15^{e}	88.00 ± 4.4^{f}	62.95 ± 3.15^{e}	65.06 ± 3.25^{de}

3	23.82± 1.19^{b}	37.19± 1.86^{b}	52.92 ± 2.65^{d}	68.99 ± 3.45^{e}
4	77.78± 3.89^{d}	83.14 ± 4.16^{d}	21.48 ± 1.07^{bc}	46.69 ± 2.33^{c}
5	70.89± 3.54^{cd}	64.00± 3.2^{c}	69.55 ± 3.48^{f}	11.95 ± 0.60^{b}
6	73.53 ± 3.68^{c}	76.01± 3.8^{d}	24.65 ± 1.23^{c}	ND
7	ND	ND	16.93 ± 0.85^{b}	ND
8	2.12 ± 0.11^{a}	8.42 ± 0.42^{a}	4.42 ± 0.22^{a}	2.55 ± 0.13^{a}

Abbreviation: ND, not determined. Values are expressed as mean ± SD. Values are expressed as mean ± SD. Different alphabetical letters at the same column mean significantly different ($P < 0.05$).

3.9 Anticancer activity

The MTT results showed the effects of *A. monosperma* and *T.hirsute* extracts on the HepG2 cell line (**Figure** 4). The extracts significantly increased cell proliferation in a dose-dependent manner, by up to 70% and 52%, respectively. The calculated IC_{50} values were 741.92 ± 102.7 ppm and 590.94 ± 61.2 ppm, respectively, compared to doxorubicin, as a standard anticancer drug with 29.67 ± 1.29 ppm, as shown in **Table** 7.

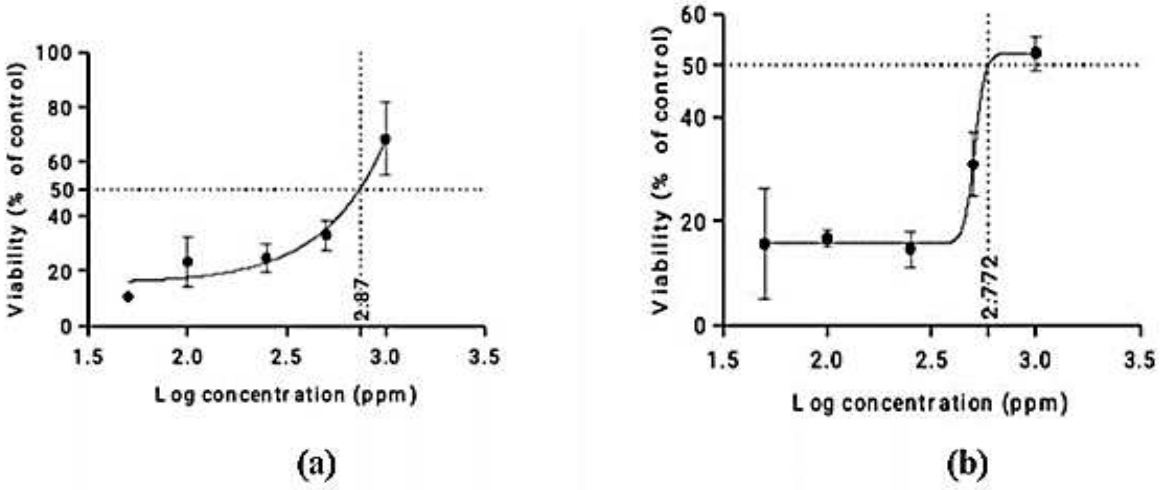


Figure 4. (a) Anticancer activity (%) of *Artemisia monosperma*; (b) Anticancer activity of *T. hirsuta.*

578 Altogether that the study of Ly, et al. [48] showed that *Artemisia vulgaris* inhibited the *in*
579 *vitro* growth of HepG2 cells with IC_{50} calculated around 100 µg/mL. This difference in the data
580 and IC_{50} may be due to the use of different plant species. Accordingly, Elhady, et al. [49] found
581 that *T. hirsuta* inhibited the growth of HepG2 cells *in vitro* with an IC_{50} calculated as 9.4 µM.
582 The extract used in the authors' studies may be collected from different areas that face extreme
583 environmental conditions, which increase the active principle compounds (as anticancer).
584
585 **Table 7**. The IC_{50} of ethanolic extracts from different samples against HepG2 cell line.

Extract/Compound	IC_{50} (ppm)
A. monosperma Delile	741.92± 102.7[b]
T. hirsuta (L.) Endl	590.94± 61.2[a]
Drug (Doxorubicin)	29.67± 1.29 [a]

Values are expressed as mean ± SD. Different alphabetical letters mean significantly different ($P < 0.05$).

586
587 A critical evaluation of the cytotoxic activity findings also suggests that the observed
588 activities may be attributed to specific phytochemicals. Such mechanistic evidence supports
589 our findings that *T. hirsuta* displayed the most potent anticancer activity among the three
590 studied plants. Moreover, the role of plant extracts in sensitizing HepG2 cells to standard
591 chemotherapeutics should not be overlooked. Al-Shafie, et al. [50] demonstrated that combining
592 natural extracts with doxorubicin can improve energy metabolism in HepG2 cells and increase
593 their susceptibility to the drug. This suggests a potential synergistic strategy where
594 phytochemicals from desert plants may serve not only as direct cytotoxic agents but also as
595 adjuvants to enhance standard chemotherapy. Taken together, these findings demonstrate that
596 while crude extracts of *A. monosperma* and *T. hirsuta* were less potent than doxorubicin, their
597 activities are biologically meaningful, particularly when considering the mechanistic roles of

598 flavonoids and phenolics. Their potential as complementary agents in anticancer therapy, either
599 as functional nutraceuticals or as sources of lead compounds, merits further investigation
600 through *in vivo* model studies.

601 *3.10 Antiviral Activity*

602 Antiviral activity testing using the MTT cytotoxicity assay showed that A. monosperma
603 exhibits moderate anti-229E activity, with an IC_{50} of 35.1 μg/mL (**Figure** 5). According to
604 Kim, et al. [51], the IC_{50} of *Artemisia annua L*. ethanolic extract against SARS-CoV BJ-001 was
605 34.5 2.6 ug/mL. Additionally, Kohn, et al. [52] determined the IC_{50} against Avian
606 Metapneumovirus (aMPV) in CER cell lines of *Aspidosperma tomentosum*, *Gaylussacia*
607 *brasiliensis*, *Virola sebifera*, and *Arrabidaea chica* that were 45.86 g/mL, 22.33 g/mL, 202
608 0.05 g/mL, and 412.7 g/mL, respectively.

609 In comparison to previous research, this study indicates that the extract of *A.*
610 *monosperma* exhibits better antiviral activity, with an IC_{50} of 35.1 μg/mL and CC_{50} of 893.22
611 μg/mL, resulting in an SI of 25.45. In an ideal situation, the higher the CC_{50} value and the lower
612 the IC_{50} value, the more potent the drug is against the virus. Orosco [53] reported that Artemisia
613 sp. and its derivatives, such as artemisinin, have shown significant antiviral activity against
614 several viruses, including SARS-CoV-2, which is related to the 229E virus. Beyond single
615 compounds, Nie, et al. [54] reported that extracts from *Artemisia* species often interfere directly
616 with viral replication and modulate host immune responses. Specific compounds from
617 *Artemisia*, such as artemisinin, have been found to inhibit viral replication by enhancing the
618 host's type I interferon response and inhibiting virus-induced inflammatory reactions [55,56].

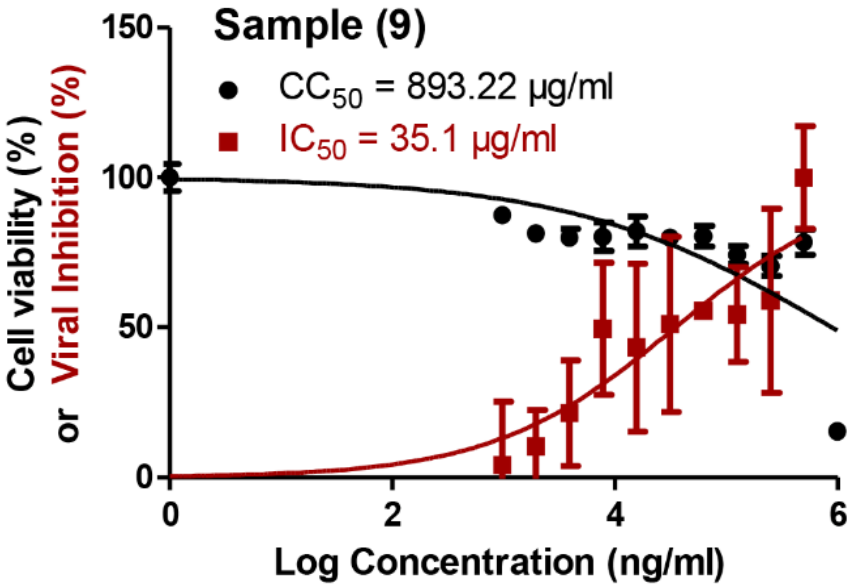


Figure 5. The IC_{50} of 229E viral activity was calculated using a nonlinear regression plot of the *A. monosperma* extract versus the normalized response (variable slope).

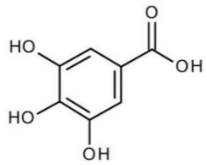
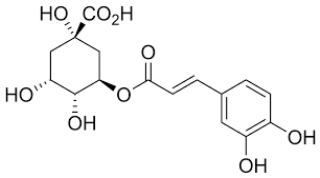
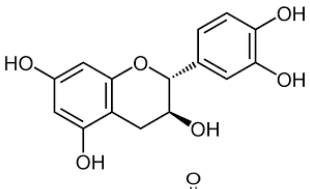
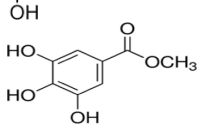
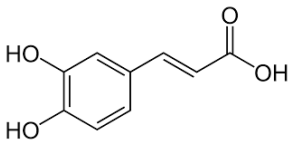
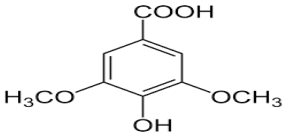
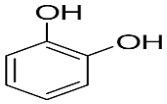
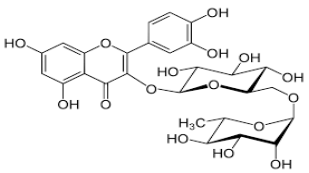
3.11 The separated compounds by HPLC contributions

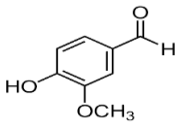
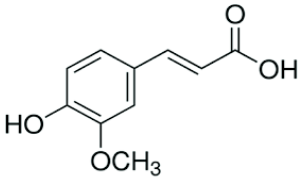
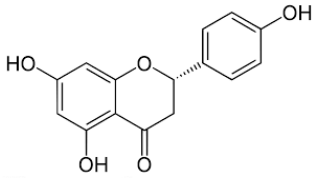
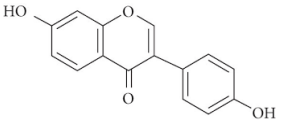
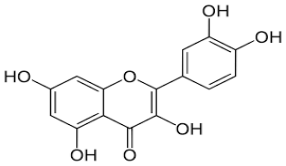
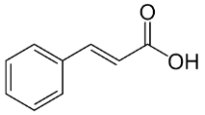
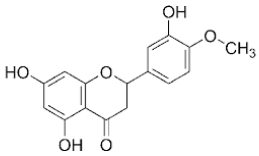
Medicinal plants have a mixture of natural compounds with several biological activities for human health, so HPLC is used to detect these components in the successive extracts of *Artemisia monosperma* and *T. hirsuta*. The compounds detected by HPLC in each extract of the tested plants, along with their biological activities, are presented in **Figures** 6 and 7, and **Tables** 8 and 9, respectively.

The results of HPLC showed that the ethanol extract of *A. monosperma* has a high concentration of quercetin, daidzein, naringenin, cinnamic acid, and hesperidin (**Figure** 6, **Table** 8). Quercetin was the major compound in the ethanolic extract, which represented 2543.40 µg/mL. This compound may be responsible for the high biological activity of the extract. The contents of daidzein, naringenin, and chlorogenic acid were 146.74, 114.23 µg/mL, and 143.99 µg/mL, respectively, and ranked as second level. These compounds may also contribute to the anticancer and antioxidant activities of the extract. Data in (**Figure** 7)

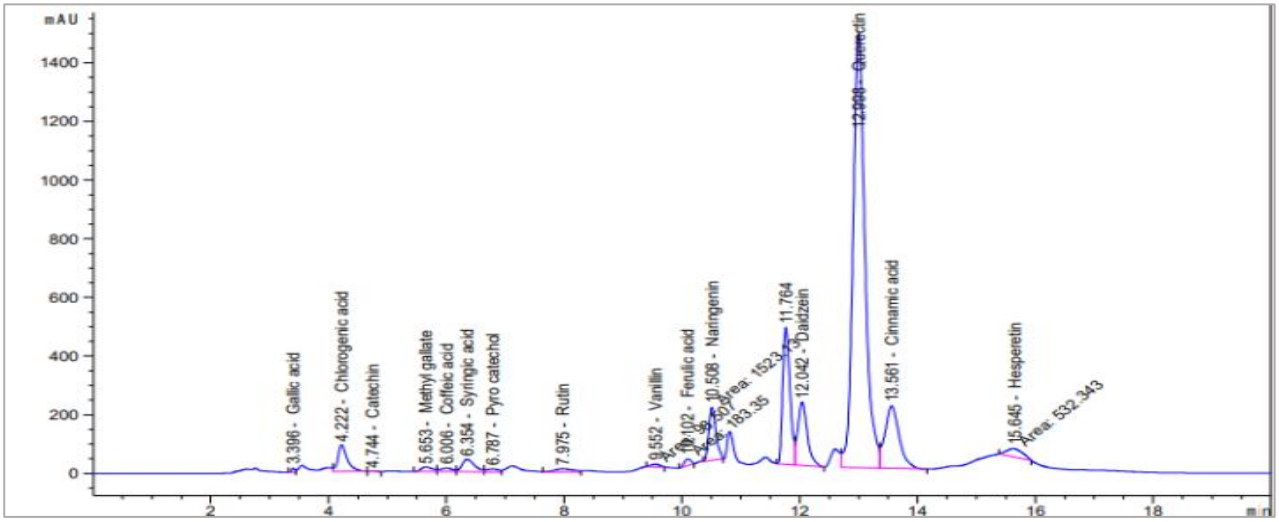
635 and (**Table** 9) show the detected compounds in *T. hirsuta* by HPLC analysis, which indicates
636 that the major compound was Chlorogenic acid (114.23 µg/ mL), which may be responsible
637 for the antioxidant and anticancer activity of the extract**.**
638

Table 8. List of phytochemical constituents (µg/g) in ethanol extract of *A. monosperma* analyzed by HPLC.

No.	RT (min)	Chemical name	Chemical structure	Conc. (µg/mL)	Biological activities	Ref.
1	3.39	Gallic acid		4.19	Antioxidant Anticancer Anti-inflammation	57
2	4.22	Chlorogenic acid		141.38	antioxidant antibacterial antitumor anti-inflammatory	58
3	4.74	Catechin		1.48	antioxidant anticancer anti-diabetic	59
4	5.65	Methyl gallate		12.18	antibacterial antioxidant anticancer anti-inflammatory	60
5	6.006	Caffeic acid		12.94	antioxidant anti-inflammatory anti-carcinogenic activity	61
6	6.35	Syringic acid		47.09	antioxidant activity	62
7	6.78	Pyrocatechol		17.33	Antimicrobial	63
8	7.97	Rutin		25.64	anti-inflammatory anti-microbial anti-tumour anti-asthma	64

9	9.55	Vanillin		2.99	Anti-carcinogenic antioxidant	65
10	10.10	Ferulic acid		9.61	anti-inflammatory antimicrobial anticancer anti-arrhythmic antithrombotic activity	66
11	10.50	Naringenin		143.99	antioxidant antitumor antiviral antibacterial	67
12	12..04	Daidzein		146.74	Antioxidant	68
13	12.99	Quercetin		2543.40	Antioxidant antibacterial antiviral	69
14	13.56	Cinnamic acid		69.05	Antioxidant Antimicrobial	70
15	15.64	Hesperetin		28.96	anti-inflammatory antioxidant antibacterial anticancer	71

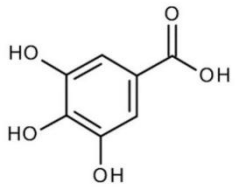
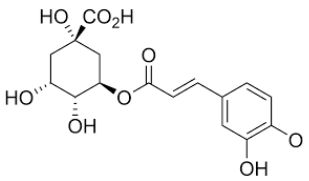
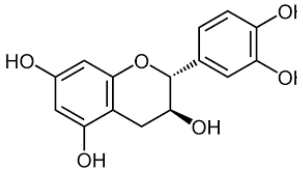
639

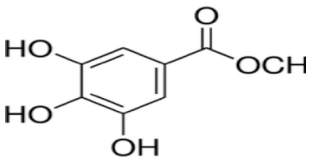
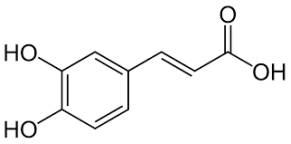
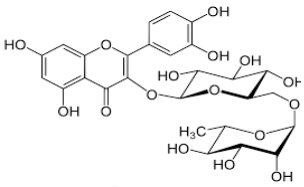
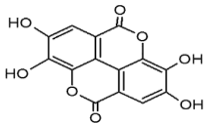
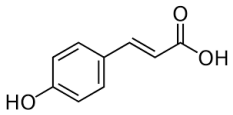
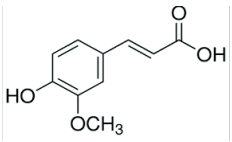
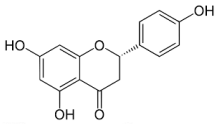
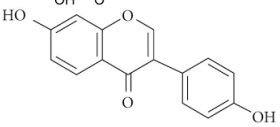
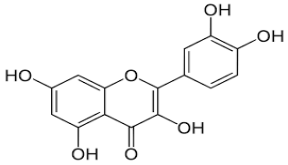
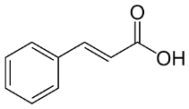


640 **Figure 6.** HPLC chromatogram of Ethanol extract from *Artemisia monosperma Delile.*

641 The results of the present study are in accordance with that found by Al-Gendy, et al. [72]
642 who reported that A. *monosperma* had high concentration of naringenin, quercetin and
643 hesperidin as 460, 850 and 1760 µg/mL respectively. Also, the previous study of Amari, et al.
644 [73] showed that chlorogenic acid occurred in a high concentration in *T. hirsuta* which agreed
645 with the current results. Thus, *S. plumosa* exhibited the highest antioxidant activity (91.77%
646 ABTS scavenging) at 100 µg/mL, surpassing other herbs. More importantly, *A.*
647 *monosperma* and *T. hirsuta* showed significant anticancer activity against HepG2 cells, with
648 IC_{50} values of 741.92 ppm and 590.94 ppm, respectively. That implication was due to the
649 quercetin (2543.40 µg/mL), naringenin (143.99 µg/mL), and chlorogenic acid (141.38 µg/mL)
650 identified in *A. monosperma*. Besides, *T. hirsuta* demonstrated moderate antiviral activity
651 against the 229E virus (IC_{50} = 35.1 µg/mL), indicating potential against viral infections.
652 Furthermore, a multi-method antioxidant validation, including DPPH, ABTS, H_2O_2, and
653 methylene blue assays, confirmed strong antioxidant capacities across all extracts.
654

Table 9. List of phytochemical constituents (µg/g) in ethanol extract of *T. hirsuta (L.) Endl* analyzed by HPLC.

No.	RT (min)	Chemical name	Chemical structure	Conc. (µg/mL)	Biological activities	Ref.
1	3.37	Gallic acid		6.57	Antioxidant Anticancer Anti-inflammation	57
2	4.22	Chlorogenic acid		114.23	antioxidant antibacterial antitumor anti-inflammatory	58
3	4.67	Catechin		9.87	antioxidant anticancer anti-diabetic	59

4	5.35	Methyl gallate		1.25	antibacterial antioxidant anticancer anti-inflammatory	60
5	5.98	Caffeic acid		9.70	antioxidant anti-inflammatory anti-carcinogenic activity	61
6	7.83	Rutin		7.86	anti-inflammatory anti-microbial anti-tumour anti-asthma	64
7	8.67	Ellagic acid		17.94	Antioxidative anti-inflammatory antibacterial	74
8	9.29	Coumaric acid		2.69	antioxidant anti-inflammatory analgesic ant-antimicrobial	75
9	10.08	Ferulic acid		1.43	anti-inflammatory antimicrobial anticancer anti-arrhythmic antithrombotic activity	66
10	10.54	Naringenin		26.97	antioxidant antitumor antiviral antibacterial	67
11	12..06	Daidzein		26.22	Antioxidant	68
12	13.04	Quercetin		17.89	Antioxidant anti-inflammatory antibacterial antiviral radical-scavenging gastro-protective immune modulatory activities	69
13	14.04	Cinnamic acid		1.08	Antioxidant Antimicrobial	70

655

656

657

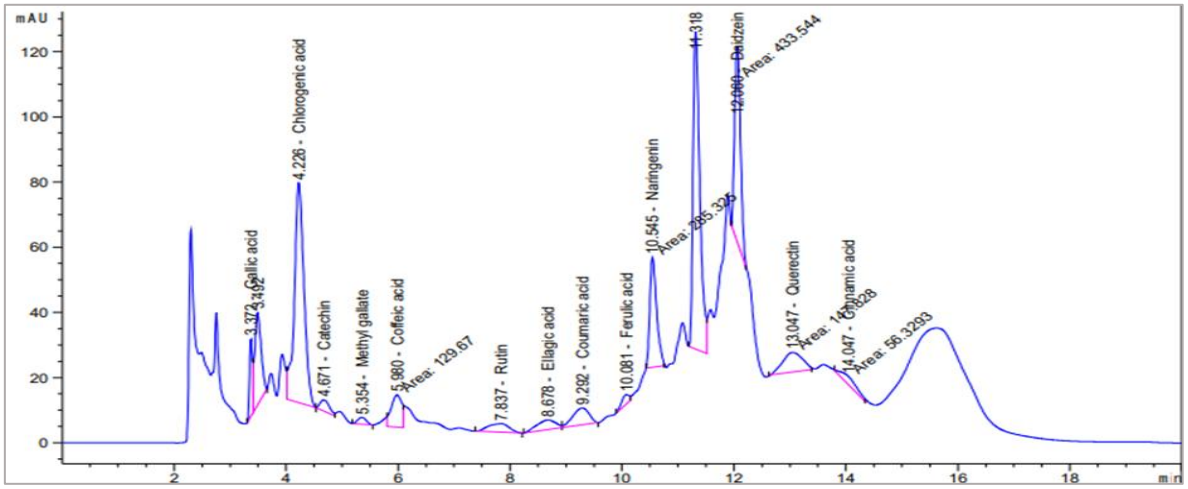


Figure 7. HPLC chromatogram of Ethanol extract from *T. hirsuta (L.) Endl.*

Although the current study used a hybrid antioxidant reaction of *S. plumosa* and *A. monosperma* extracts with ascorbic acid, it enhanced their efficacy (69.59% and 67.03% activity). Its limitation came from its need for more comprehensive quantitative screening to confirm the integration of phenolics, flavonoids, coumarins, and fatty acids, highlighting the plants' therapeutic clinical potential. These studies could focus on *T. hirsuta*, which contained the highest phenolic (1520 mg/100g) and flavonoid (223 mg/100g) content, correlating with its bioactivities. In addition, *A. monosperma's* ethanol extract yielded potent antiradical fractions (e.g., Fraction 1: 90.29% ABTS scavenging) and contained 540 mg/100g ascorbic acid, higher than *S. plumosa* (60 mg/100g).

The observed antiviral activity, particularly for *A. monosperma* (IC_{50} = 35.1 µg/mL; SI = 25.45), may result from multiple complementary mechanisms related to quercetin and naringenin. Roy, et al. [76] reported that quercetin blocks viral entry by disrupting the spike–ACE2-mediated syncytium formation that interferes with furin-mediated spike processing. Molecular docking study by Derosa, et al. [77] suggested that quercetin inhibits coronavirus replication via targeting proteases (e.g., 3CLpro with binding energy −6.25 kcal/mol and PLpro with binding energy −4.62 kcal/mol). At the same time, it is significantly inhibits RdRp (IC_{50}

≈ 6.9 μM) [78]. In addition, chlorogenic acid and caffeic acid in *T. hirsute* may contribute to its immunomodulation by inhibiting NF-κB signaling and modulating innate immune sensors, such as MDA5 and TLR7 [79,80]. Together, these entry, replication, and host-directed effects provide a plausible mechanistic rationale for the broad-spectrum antiviral activity of the extracts in the current study.

4. Conclusions

The leaves and stem of *S. plumosa (L.) Munro ex T. Anderson*, *A. monosperma* and *T. hirsuta (L.) Endl* were evaluated in the current study as a valuable natural source of antioxidant activity as well as other potential biological activities (anticancer and antiviral). Successive hexane, ethyl acetate, and ethanol 70% extracts were evaluated and showed that ethanol 70% extract is the most promising extract. Ethanol 70% showed a high antioxidant activity against methylene blue, DPPH, and ABTS, respectively, as well as the highest activity in TLC bio-autography among other extracts. In addition, the highest antioxidant activity against ABTS was observed in fractions 1, 2, 4, and 6, as well as against methylene blue. The extract exhibited a slight cytotoxic effect against the HepG2 cell line, as determined by an MTT cytotoxicity assay. Furthermore, the ethanol extract of *A. monosperma* has shown potential as an antiviral agent. The promising biological activity of the extract may be attributed to its high phenolic and flavonoid content, as well as other secondary metabolites, as confirmed by HPLC analysis. Consequently, it is recommended to use these extracts as natural antioxidants and antiviral compounds, as they may be a better alternative to synthetic ones, benefiting the functional foods, supplements, and pharmaceutical industries.

Ethics approval and consent to participate

No ethical approval was required in the current study.

Competing interests

The authors declare that they have no competing interests.

5. Authors Contributions

Conceived and designed the experiments: EAS, AMA, MK. Performed the experiments: SME, NRA, and AEE. Collection of data: SME, NRA, MMG, RSH, and AEE. Analyzed the data: SME, MMG, NRA, EAS, AMA, MK, and AEE. Contributed reagents/materials/analysis tools: SME, NRA, EAS, AMA, MK, and AEE. Write the original draft: SME, NRA, MMG, RSH, EAS, AMA, MK, and AEE. Revising of the manuscript: SME, MMG, RSH, EAS, AMA, MK, and AEE.

Funding Sources

No specific fund has been received for the present study.

Clinical trial number

Not applicable.

Consent for publication

All authors read and approved the final manuscript.

Availability of data and materials

All the raw data used and/or analyzed during this study are available from the corresponding author (Prof. Mostafa M. Gouda) upon reasonable request.

Reference

1 Omar, H. H., Al-Judaibiand, A. & El-Gendy, A. Antimicrobial, antioxidant, anticancer activity and phytochemical analysis of the red alga, Laurencia papillosa. *International Journal of Pharmacology* **14**, 572-583 (2018).

2 Elsharkawy, E. R. *et al.* The antidiabetic, haematological, and antioxidant implications of Schimpera arabica natural plant on Streptozotocin-diabetic rats. *Journal of Agriculture and Food Research*, 101891 (2025).

3 Mallik, S. *et al.* The positive implication of natural antioxidants on oxidative stress-

mediated diabetes mellitus complications. *Journal, genetic engineering & biotechnology* **22**, 100424, doi:10.1016/j.jgeb.2024.100424 (2024).

4 Villanueva, A. Hepatocellular Carcinoma. *N Engl J Med* **380**, 1450-1462, doi:10.1056/NEJMra1713263 (2019).

5 Sander, W. J. *et al.* Reactive oxygen species as potential antiviral targets. *Reviews in Medical Virology* **32**, e2240 (2022).

6 Abdulaziz, H. A. *et al.* The prevalence and associated factors of viral hepatitis and cryptogenic related hepatocellular carcinoma at King Abdulaziz Medical city-Riyadh. (2019).

7 Pandey, K. B. & Rizvi, S. I. Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative medicine and cellular longevity* **2**, 270-278, doi:10.4161/oxim.2.5.9498 (2009).

8 Lv, J. M., Gouda, M., Zhu, Y. Y., Ye, X. Q. & Chen, J. C. Ultrasound-Assisted Extraction Optimization of Proanthocyanidins from Kiwi (Actinidia chinensis) Leaves and Evaluation of Its Antioxidant Activity. *Antioxidants (Basel, Switzerland)* **10**, doi:10.3390/antiox10081317 (2021).

9 Gouda, M. M., Elsharkawy, E. R., He, Y. & Li, X. Importance of Advanced Detection Methodologies from Plant Cells to Human Microsystems Targeting Anticancer Applications. **26**, 4691 (2025).

10 Liu, W. *et al.* Quercetin induced HepG2 cells apoptosis through ATM/JNK/STAT3 signaling pathways. *Biocell* **47**, 187-194 (2022).

11 Motallebi, M. *et al.* Naringenin: A potential flavonoid phytochemical for cancer therapy. *Life Sciences* **305**, 120752 (2022).

12 Kolev, E. *et al.* Echinacea Purpurea For the Long-Term Prevention of Viral Respiratory Tract Infections During Covid-19 Pandemic: A Randomized, Open, Controlled,

751 Exploratory Clinical Study. *Front Pharmacol* **13**, 856410, doi:10.3389/fphar.2022.856410
752 (2022).
753 13 Abdelghany, T. *et al.* Effect of Thevetia peruviana seeds extract for microbial pathogens
754 and cancer control. (2021).
755 14 Waweru, D. M., Arimi, J. M., Marete, E., Jacquier, J.-C. & Harbourne, N. Current status
756 of utilization and potential of Dovyalis caffra fruit: major focus on Kenya-a review.
757 *Scientific African* **16**, e01097 (2022).
758 15 Bawazeer, M., Orabi, M. A., Yaseen, M. & Abdelkader, M. S. A. Cytotoxic Potential of
759 Phenolic Glycosides from Stipagrostis plumosa. *Revista Brasileira de Farmacognosia*, 1-
760 6 (2021).
761 16 Abu-Niaaj, L. & Katampe, I. Isolation and Characterization of Flavones from Artemisia
762 monosperma. *Pharmacognosy Journal* **10** (2018).
763 17 El-Sherbeny, G. A., Dakhil, M. A., Eid, E. M. & Abdelaal, M. Structural and chemical
764 adaptations of Artemisia monosperma Delile and Limbarda crithmoides (L.) Dumort. in
765 response to arid coastal environments along the mediterranean coast of Egypt. *Plants* **10**,
766 481 (2021).
767 18 Alkaraki, A. K., Aldmoor, M. A., Lahham, J. N. & Awad, M. DNA barcoding of two
768 Thymelaeaceae species: Daphne mucronata Royle and Thymelaea hirsuta (L.) Endl. *Plants*
769 **10**, 2199 (2021).
770 19 Pvman, F. The chemical investigation of plants. By Dr. L. Rosenthaler. Translated from the
771 German by Dr. S. Ghosh. Monographs on Modern Chemistry. Edited by Prof. J. Kendall,
772 MA, D. Sc., FRS, and Prof. J. Read, MA, B. Sc., Ph. D. Pp. viii+ 197. London: G. Bell &
773 Sons, Ltd., 1930. 12s. 6d. *Journal of the Society of Chemical Industry* **50**, 356-356 (1931).
774 20 Shaikh, J. R. & Patil, M. Qualitative tests for preliminary phytochemical screening: An
775 overview. *International Journal of Chemical Studies* **8**, 603-608 (2020).

21 Hernández-López, A. *et al.* Quantification of reducing sugars based on the qualitative technique of Benedict. *ACS omega* **5**, 32403-32410 (2020).

22 Gouda, M., Chen, K., Li, X., Liu, Y. & He, Y. Detection of microalgae single-cell antioxidant and electrochemical potentials by gold microelectrode and Raman micro-spectroscopy combined with chemometrics. *Sensors and Actuators B: Chemical* **329**, 129229 (2021).

23 Gouda, M., Nassarawa, S. S., Gupta, S. D., Sanusi, N. I. & Nasiru, M. M. Evaluation of carbon dioxide elevation on phenolic compounds and antioxidant activity of red onion (Allium cepa L.) during postharvest storage. *Plant physiology and biochemistry : PPB* **200**, 107752, doi:10.1016/j.plaphy.2023.107752 (2023).

24 Cai, L. Thin layer chromatography. *Current Protocols Essential Laboratory Techniques* **8**, 6.3. 1-6.3. 18 (2014).

25 Singleton, V. L. & Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture* **16**, 144-158 (1965).

26 Zhishen, J., Mengcheng, T. & Jianming, W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food chemistry* **64**, 555-559 (1999).

27 Hayat, J. *et al.* Phytochemical screening, polyphenols, flavonoids and tannin content, antioxidant activities and FTIR characterization of Marrubium vulgare L. from 2 different localities of Northeast of Morocco. *Heliyon* **6** (2020).

28 Belyagoubi-Benhammou, N., Belyagoubi, L., El Zerey-Belaskri, A. & Atik-Bekkara, F. In vitro antioxidant properties of flavonoid fractions from Pistacia atlantica Desf. subsp. atlantica fruit using five techniques. *J Mech Eng Sci* **6**, 1118-1125 (2014).

29 Vahid, B. Titrimetric determination of ascorbic acid contents in plant samples by 2, 6-

dichlorophenolindophenol method. *Journal of the Chemical Society of Pakistan* **34**, 1510-1512 (2012).

30 Gaber, N. B., El-Dahy, S. I. & Shalaby, E. A. Comparison of ABTS, DPPH, permanganate, and methylene blue assays for determining antioxidant potential of successive extracts from pomegranate and guava residues. *Biomass Conversion and Biorefinery*, 1-10 (2021).

31 Dandare, S., Ezeonwumelu, I., Ezeh, C. & Auta, H. Determination of in vitro antioxidant and radical scavenging activities of different extracts of Allium sativum (Garlic). *J Pharm Biol Sci* **9**, 69-73 (2014).

32 Younes, K. M. *et al.* In-vitro evaluation of antioxidant and antiradical potential of successive extracts, semi-purified fractions and biosynthesized silver nanoparticles of Rumex vesicarius. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* **49**, 12293-12293 (2021).

33 Gouda, M. *et al.* Bioprobe-RNA-seq-microRaman system for deep tracking of the live single-cell metabolic pathway chemometrics. *Biosensors and Bioelectronics* **261**, 116504, doi:*https://doi.org/10.1016/j.bios.2024.116504* (2024).

34 Hansen, M. B., Nielsen, S. E. & Berg, K. Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. *Journal of immunological methods* **119**, 203-210 (1989).

35 Mostafa, A. *et al.* FDA-approved drugs with potent in vitro antiviral activity against severe acute respiratory syndrome coronavirus 2. *Pharmaceuticals* **13**, 443 (2020).

36 Khalil, S. R. *et al.* Dual immunological and oxidative responses in Oreochromis niloticus fish exposed to lambda cyhalothrin and concurrently fed with Thyme powder (Thymus vulgaris L.): Stress and immune encoding gene expression. *Fish & shellfish immunology* **100**, 208-218 (2020).

37 Amari, N. O., Bouzouina, M., Berkani, A. & Lotmani, B. Phytochemical screening and

826 antioxidant capacity of the aerial parts of Thymelaea hirsuta L. *Asian Pacific journal of*
827 *tropical disease* **4**, 104-109 (2014).
828 38 Ismail, Hammouda, F., Rizk, A. & Hassan, N. Methylated flavonoids from Artemisia
829 monosperma. (1989).
830 39 Abdelkader, M. S. A., Orabi, M. A., Shama, S. M. & Assaf, M. H. Chemical constituents
831 and biological activities of the aerial parts of Stipagrostis plumosa (L) Munro ex T.
832 Anderson. *Journal of Pharmaceutical Sciences and Research* **8**, 801 (2016).
833 40 Zayyat, M., Khedr, A.-H. & Amer, N. Phytochemical and antimicrobial activity of two
834 Egyptian xerophytes: Artemisia jaudica L. and Artemisia monosperma Delile. *African*
835 *Journal of Biological Sciences* **14**, 127-140 (2018).
836 41 Trigui, M., Hsouna, A. B., Tounsi, S. & Jaoua, S. Chemical composition and evaluation of
837 antioxidant and antimicrobial activities of Tunisian Thymelaea hirsuta with special
838 reference to its mode of action. *Industrial Crops and Products* **41**, 150-157 (2013).
839 42 El-Beltagi, H. S., Mohamed, H. I., Megahed, B. M., Gamal, M. & Safwat, G. Evaluation
840 of some chemical constituents, antioxidant, antibacterial and anticancer activities of Beta
841 vulgaris L. root. *Fresenius Environmental Bulletin* **27**, 6369-6378 (2018).
842 43 Fatima, I., Kanwal, S. & Mahmood, T. Microbiostatic, antioxidative and cytotoxic
843 potentiation of some grasses of Bahawalpur, Pakistan. *Journal of Traditional Chinese*
844 *Medicine* **39**, 482 (2019).
845 44 Babu, B., Shylesh, B. & Padikkala, J. Antioxidant and hepatoprotective effect of Acanthus
846 ilicifolius. *Fitoterapia* **72**, 272-277 (2001).
847 45 Merghem, M., Dahamna, S. & Khennouf, S. Polyphenols contents and antioxidant Activity
848 of extracts from Leaves and flowers of Thymelaea hirsuta. *Journal of Drug Delivery and*
849 *Therapeutics* **10**, 108-111 (2020).
850 46 Al-Soqeer, A. Antioxidant activity and biological evaluation of hot-water extract of

Artemisia monosperma and Capparis spinosa against lead contamination. *Research journal of Botany* **6**, 11 (2011).

47 Laouini, S., Kelef, A. & Ouahrani, M. Free radicals scavenging activity and phytochemical composition of astermisia (Herba-Alba) extract growth in Algeria. *Journal of Fundamental and Applied Sciences* **10**, 268-280 (2018).

48 Ly, B. T. K. *et al.* Screening of medicinal herbs for cytotoxic activity to leukemia cells. *J. Buon* **25**, 1989-1996 (2020).

49 Elhady, S. S. *et al.* New antiproliferative triflavanone from Thymelaea hirsuta—Isolation, structure elucidation and molecular docking studies. *Molecules* **26**, 739 (2021).

50 Al-Shafie, T. A. *et al.* A Proposed Association between Improving Energy Metabolism of HepG2 Cells by Plant Extracts and Increasing Their Sensitivity to Doxorubicin. *Toxics* **11**, doi:10.3390/toxics11020182 (2023).

51 Kim, H.-Y. *et al.* Medicinal herbal extracts of sophorae radix ACANTHOPANACIS cortex sanguisorbae radix and Torilis fructus inhibit coronavirus replication in vitro. *Antiviral therapy* **15**, 697-709 (2010).

52 Kohn, L. *et al.* In-vitro antiviral activities of extracts of plants of the Brazilian cerrado against the avian metapneumovirus (aMPV). *Brazilian Journal of Poultry Science* **17**, 275-280 (2015).

53 Orosco, F. L. Current Advances in Antiviral Potential of <italic>Artemisia</italic> Against Major Viral Infections. *JOURNAL OF BACTERIOLOGY AND VIROLOGY* **53**, 61-73, doi:10.4167/jbv.2023.53.2.061 (2023).

54 Nie, C. *et al.* In vitro efficacy of Artemisia extracts against SARS-CoV-2. *Virology Journal* **18**, 182, doi:10.1186/s12985-021-01651-8 (2021).

55 Ji, O. *et al.* Artemisia and Artemisia-based products for COVID-19 management: current state and future perspective. *Adv Tradit Med* **23**, 85-96 (2023).

56 Orege, J. I. *et al. Artemisia and Artemisia-based products for COVID-19 management: current state and future perspective*. (ADV TRADIT MED (ADTM). 2023;23(1):85-96. doi: 10.1007/s13596-021-00576-5. Epub 2021 May 5.).

57 Kahkeshani, N. *et al.* Pharmacological effects of gallic acid in health and diseases: A mechanistic review. *Iranian journal of basic medical sciences* **22**, 225 (2019).

58 Wang, L. *et al.* The biological activity mechanism of chlorogenic acid and its applications in food industry: A review. *Frontiers in Nutrition* **9**, 943911 (2022).

59 Isemura, M. Vol. 24 528 (MDPI, 2019).

60 Kang, O.-H. *et al.* Antibacterial activity of methyl gallate isolated from Galla Rhois or carvacrol combined with nalidixic acid against nalidixic acid resistant bacteria. *Molecules* **14**, 1773-1780 (2009).

61 Espíndola, K. M. M. *et al.* Chemical and pharmacological aspects of caffeic acid and its activity in hepatocarcinoma. *Frontiers in oncology* **9**, 541 (2019).

62 Srinivasulu, C., Ramgopal, M., Ramanjaneyulu, G., Anuradha, C. & Kumar, C. S. Syringic acid (SA)–a review of its occurrence, biosynthesis, pharmacological and industrial importance. *Biomedicine & Pharmacotherapy* **108**, 547-557 (2018).

63 Kocaçalışkan, I., Talan, I. & Terzi, I. Antimicrobial activity of catechol and pyrogallol as allelochemicals. *Zeitschrift für Naturforschung C* **61**, 639-642 (2006).

64 Gullon, B., Lú-Chau, T. A., Moreira, M. T., Lema, J. M. & Eibes, G. Rutin: A review on extraction, identification and purification methods, biological activities and approaches to enhance its bioavailability. *Trends in food science & technology* **67**, 220-235 (2017).

65 Arya, S. S., Rookes, J. E., Cahill, D. M. & Lenka, S. K. Vanillin: A review on the therapeutic prospects of a popular flavouring molecule. *Advances in traditional medicine*, 1-17 (2021).

66 Zduńska, K., Dana, A., Kolodziejczak, A. & Rotsztejn, H. Antioxidant properties of ferulic

acid and its possible application. *Skin pharmacology and physiology* **31**, 332-336 (2018).

67 Salehi, B. *et al.* The therapeutic potential of naringenin: a review of clinical trials. *Pharmaceuticals* **12**, 11 (2019).

68 Dwiecki, K., Neunert, G., Polewski, P. & Polewski, K. Antioxidant activity of daidzein, a natural antioxidant, and its spectroscopic properties in organic solvents and phosphatidylcholine liposomes. *Journal of Photochemistry and Photobiology B: Biology* **96**, 242-248 (2009).

69 Kim, J. K. & Park, S. U. Quercetin and its role in biological functions: An updated review. *EXCLI journal* **17**, 856 (2018).

70 Sova, M. Antioxidant and antimicrobial activities of cinnamic acid derivatives. *Mini reviews in medicinal chemistry* **12**, 749-767 (2012).

71 Zhong, Q. *et al.* Satellite-based evaluation of AeroCom model bias in biomass burning regions. *Atmospheric Chemistry and Physics* **22**, 11009-11032 (2022).

72 Al-Gendy, A. A., Ali, R. M., Hamdan, D. I. & Abdel-Ghani, A. E.-S. Elicitation induced flavonoids, phenolic constituents, antioxidant and cytotoxic activities of Artemisia monosperma callus cultures. *Journal of Medicinal Plants Research* **10**, 717-731 (2016).

73 Amari, N. O. *et al.* Antifungal and antiaging evaluation of aerial part extracts of Thymelaea hirsuta (L.) endl. *Natural Product Communications* **16**, 1934578X20987932 (2021).

74 Vattem, D. & Shetty, K. Biological functionality of ellagic acid: a review. *Journal of food biochemistry* **29**, 234-266 (2005).

75 Aldaba-Muruato, L. R. *et al.* Therapeutic perspectives of p-coumaric acid: Anti-necrotic, anti-cholestatic and anti-amoebic activities. *World Academy of Sciences Journal* **3**, 1-8 (2021).

76 Roy, A. V. *et al.* Quercetin inhibits SARS-CoV-2 infection and prevents syncytium formation by cells co-expressing the viral spike protein and human ACE2. *Virol J* **21**, 29,

doi:10.1186/s12985-024-02299-w (2024).

77 Derosa, G., Maffioli, P., D'Angelo, A. & Di Pierro, F. A role for quercetin in coronavirus disease 2019 (COVID-19). *Phytotherapy research : PTR* **35**, 1230-1236, doi:10.1002/ptr.6887 (2021).

78 Munafò, F. *et al.* Quercetin and luteolin are single-digit micromolar inhibitors of the SARS-CoV-2 RNA-dependent RNA polymerase. *Scientific Reports* **12**, 10571, doi:10.1038/s41598-022-14664-2 (2022).

79 Abaidullah, M. *et al.* Chlorogenic acid is a positive regulator of MDA5, TLR7 and NF-κB signaling pathways mediated antiviral responses against Gammacoronavirus infection. *International Immunopharmacology* **96**, 107671 (2021).

80 Nguyen, V., Taine, E. G., Meng, D., Cui, T. & Tan, W. Chlorogenic Acid: A Systematic Review on the Biological Functions, Mechanistic Actions, and Therapeutic Potentials. **16**, 924 (2024).