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# Characterization of Chemical Constituents of *Adansonia digitata* L. using GC-MS and LC-MS/QTOF and their *In Vitro* Anti-cervical Cancer Effects

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ABSTRACT

Plants and their chemical constituents have been utilized in the development of new medicines for diseases of public health importance like cervical cancer.This study was aimed at the characterization of chemical constituents of different parts of *Adansonia digitata* and their anti-cervical cancer activity. The chemical constituents of the fruits pulp, leaves, stem bark, and root extracts of the plant were identified using GC-MS and LC-MS/QTOF techniques while the anti-cervical cancer activity of the extracts was investigated using MTT assay. The GC-MS analysis indicated the presence of 4, 12, 10, and 7 chemical compounds for the fruits pulp, leaves, stem bark, and roots, respectively. While the LC-MS/QTOF analysis revealed the presence of 10, 12, 15, and 17 compounds in the fruits pulp, roots, leaves, and stem-bark, respectively. The major phenolic compounds identified were rutin, quercetin-3-*β*-d-glucoside, gentisic acid, catechin, and diosmin. The highest amount of phenolic constituent present in the extracts was rutin (0.3192 mg/g of dry plant material) in the leaves. The extracts of *A. digitata* (50, 100, and 250 µg/mL) have shown a significant cytotoxic effect on *the HeLa* cell line in a concentration-dependent manner. Interestingly, the leaves at the highest concentration showed an excellent cytotoxic effect with 91.5, 90.8, 85.0, and 82.4% growth inhibition at 12, 24, 36, and 48 hours and $IC_{50}$ values of 99.17, 104.41, 162.23, and 170.02 µg/mL, respectively. This study indicated the promising potentials of *A. digitata* leaves as a good source of cytotoxic compounds against cervical cancer for future applications in pharmaceutical industries.

***Keywords*:***Adansoniadigitata*; anti-cervical cancer; GC-MS; LC-MS/QTOF

## Introduction

Cervical cancer is the fourth most frequent form of malignancy in women worldwide, and it remains a leading cause of cancer-related death for women in developing countries.[1] In the year 2018, there were 569, 847 new cases of cervical cancer which are accompanied by 311, 365 death.[2]Cervical cancer treatment options include surgery, radiotherapy, chemotherapy, or combinations of these which are accompanied by many side effects. Thus, there is an urgent need for a continuous search for more potent, safer, less expensive, and readily available anticancer agents.

Since time immemorial, medicinal plants have been used for the treatment and prevention of diseases, particularly in developing countries where access to healthcare facilities is low.[3]*A. digitata* L. (Family: Malvaceae) commonly known as baobab is a large deciduous tree, native to Africa. The whole plant is widely distributed in the thorn woodlands of African savannahs.[4] It has been known as a multi-purpose tree due to its widespread usage as food, medicine, nutrition, shelter, and raw materials, among others.[5]Different parts of *A. digitata* (leaves, roots, stems, fruits, seeds) are employed in Ethnomedicine in many African countries.

For instance, in Cameroon, Central African Republic, and South Africa its fruits and seeds are mixed with water to cure malaria, diarrhea, dysentery and fever.[6]

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In Sierra Leon, the leaves are used to treat diarrhea, kidney and bladder diseases, inflammation, malaria, asthma, and fever.[7]In Nigeria and Sudan, aqueous extracts from the leaves, barks, roots, and stems are used to treat anemia and malaria.[8,9] Tanzanian ethnic groups used decoction from the fruits to treat microbial infections.[10]

In terms of chemical compositions, different classes of compounds have been identified from various parts of the plant which include alkaloids, flavonoids, vitamins, steroids, lipids, carbohydrates, terpenes, saponins, phenolics, and amino acids.[11-13]

Pharmacologically, *A. digitata*has been reported to possess antimicrobial, antiviral, anti-diarrhoeal, antimalarial, antioxidant, analgesic, antipyretic, anti-insecticidal, antibacterial, hepatoprotective, inhibitory effects against *Trypanosoma* and anti-inflammatory properties.[14,15]The antitumor effect of the seeds and fruits pulp of the plant against Ehrlic *Ascites Carcinoma* was documented earlier by Elsaid.[16]Recently, Kadam and Kondawar reported the anticancer effects of the fruit against three human cancer cell lines including MCF-7, Hep-G2 and COLO-205 for breast, liver, and colon cancers, respectively.[17]

To the best of our knowledge, there is only a little information on the characterization of the constituents from *A. digitata* extracts while there is no information demonstrating the anti-cervical cancer activity of the plant. Therefore, this research was aimed atthe characterization of chemical constituents of the different parts (fruits pulp, leaves, stem bark, and roots) of *A. digitata* using GC-MS and Liquid Chromatography coupled to Quadrupole Time of Flight Mass Spectrometry (LC-MS/QTOF) and investigate their anti-cervical cancer potential.

## Materials and Methods

*Cell line and Reagents*

Trypan blue (Hyclone, Lot no: JRH27098), Sodium bicarbonate (MP Biomedicals, Lot No: 2048J), EDTA (MP Biomedicals, Lot No: 6941H), DPBS (Dulbecoo's phosphate buffer saline) (MP Biomedicals,

Lot No: C1290), Trypsin (Invitrogen, Lot No: 1376596), SRB Dye, MTT Salt. MTT (Roche applied sciences, Cat. No. 11465 007 001), DMEM (Dulbecoo's Modified Eagels medium, high glucose), DMEM (Dulbecco's Modified Eagels medium, low glucose), FBS (Fetal Bovine Serum) (Bioclot, Lot No: 07310).

*Plant Material*

The roots, stem-bark, leaves, and fruits pulp of *A. digitata* were collected in July 2022 from AI-Fashir, North Darfur State, Sudan. They were identified by the botanist of the Medicinal and Aromatic Plants and Traditional Medicinal Research Institute, National Center for Research (NCR), Khartoum, Sudan where a herbarium specimen (No 1579) was deposited.

*Extraction and Preparation of Extracts*

The different parts of *A. digitata* were properly washed, shade-dried and pulverized to powder using a mechanical grinder(RH 2000, Retsh GmbH, Germany).Exactly50 g of powdered samples each were macerated separately with ethanol at room temperature overnight with occasional shaking and stirring. The resulting extracts were filtered with Whatman no. 1 filter paper and concentrated to dryness using a rotary evaporator (R215, Switzerland)and further dried on Petri dishes under open air.[18]The stock solution of each extract (200 ppm) was filtered through 0.22 µm polyether sulfone (PES) syringe filters (Jiangsu Green Unit Science Instrument) and used for the GC-MS and LC-MS/QTOFanalysis.

*GC-MS Analysis*

The fatty acids present in different parts of *A. digitata* extracts were identified using a GC-MS device which is composed of Agilent technologies mass spectroscopy detector and Agilent technologies 7890A gas chromatography. Chromatographic analysis was achieved using a DB-1 capillary column (30 m x 0.25 mm id, film thickness 0.25 µm) and the separation was performed at a flow rate of 1.5 mL/min with helium as the carrier gas. The temperature began at 60 ºC for two min, it was increased at the rate of 4 ºC/min within 15 min and was finally maintained at 200 ºC for 20 min. The temperature of the injector was maintained at 240 ºC and the mass range scanned was 3-500 *m/*z.[19]The identification of the constituents was based on the comparison of their mass spectra with those in the system's spectral library.

*LC-MS/QTOF Analysis of Phenolic Compounds in A. digitata Extracts*

LC-MS/QTOF analysis was used to characterize the phenolic constituents present in the different parts of *A. digitata*extracts (roots, stem-bark, leaves, and fruits pulp). LC-MS consists of autosampler Sil-10ADvp (Shimadzu, Japan), CTO-20A HPLC, and 2LC-10ADvp pumps. Chromatographic isolation was carried out using an Agilent shim-pack VP-ODS column (150 mm × 4.6 mm i.d.,1.8 µm). The elution was performed by gradient solvent systems with a flow rate of 1 mL/min at 25 ºC. The mobile phase consisted of 70% water (solvent A) and 30% acetonitrile (solvent B) changed gradually to 90% acetonitrile within 25 min and held for another five min. The total running time was 30 min and the sample injection volume was 10 µL of the solution, while the wavelength of the UV-Vis detector was set at 230 nm.[20]LC system was coupled with a 6210 Time of Flight (TOF) mass spectrometer equipped with an electro-spray ionization (ESI) source was used to perform the MS analysis. The identification of the constituents was based on the comparison of their mass spectra with those in the system's spectral library.

*Cytotoxicity Assay*

*Microculture Tetrazolium (MTT) assay*

Cytotoxicity assay on cervical cancer cell line (*HeLa*) was conducted by a methodpreviously reported elsewhere with slight modifications.[21] The monolayer cell culture was trypsinized and the cell count was adjusted to 3-lakh cells/mL using a medium containing 10% newborn calf serum. To each well of 96-well microtitre plates, 0.1 mL of diluted cell suspension was added. After 24 hours, when the monolayer was formed, the supernatant was flicked off and 100 µL of different concentrations (250–50 µg/mL) of different extracts were added to the cells in microtitre plates. They were kept for incubation at 37 ºC in a 5% $CO_2$incubator(Unimax 1010, Germany) for 72 hours. The cells were periodically checked for granularity, shrinkage, and swelling. After 72 hours, the sample solution in wells was flicked off and 50 µL of MTT dye was added to each well. The plates were gently shaken and incubated for 4 hours at 37 ºC in a 5% $CO_2$ incubator. The supernatant was removed, 50 µL of propanol was added, and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader (BT-CA20, China) at a wavelength of 490 nm at different hours (12, 24, 36, and 48). The percentage growth inhibition was calculated.

*Data Interpretation*

The decrease in the rate of cell proliferation was determined via the absorbance values that were lower than the control. Conversely, a higher absorbance rate indicates an increase in cell proliferation. The percentage growth survival and inhibition were calculated via the following formulas:

$$\%\ Cell\ survival = \frac{Absrobance(test) - Absorbance\ (blank)}{Absorbance(control) - Absorbance\ (blank)} \text{ X } 100$$

$$\%\ Cell\ inhibition = 100 - Cell\ survival$$

## Results and Discussion

*GC-MS Analysis*

The GC-MS analysis of the different parts of *A. digitata* extracts indicated the presence of 4, 12, 10, and 7 chemical compounds for the fruits pulp, leaves, stem-bark, and roots, respectively [Table 1 and Figure 1]. The predominant compound presented in the different plant part extracts was palmitic acid, with relative abundances of 48.78% (fruits pulp), 37.21% (stem-bark), 30.53% (roots), and 8.95% (leaves).

*LC-MS/QTOF Analysis of Phenolic Compounds in A. digitata Extracts*

Figure 2 shown the spectrum of compounds detected from the LC-MS/QTOF analysis. The high-resolution of QTOF-MS identified the presence of 22 compounds, including 10 phenolic acids (gentisic acid, 4-hydroxybenzoic acid, syringic acid, vanillic acid, cinnamic acid, protocatechuic acid, chlorogenic acid, sinapic acid, caffeic acid and gallic acid), 10 flavanoids (catechin, morin, diosmin, naringin, rutin, scutellarin, quercetin-3-*β*-d-glucoside, apigetrin, neohesperidin and apigenin), one stilbene glycoside (polydatine) and one dicarboxylic acid (fumaric acid) [Table 2].

*Cytotoxicity Assay of A. digitata Extracts*

Cytotoxicity studies of the different parts (fruits pulp, leaves, stem-bark, and roots) of *A. digitata* were conducted using *HeLa* cell line at different concentrations and different times intervals ranging from 12–48 hours to determine the $IC_{50}$ (50% growth inhibition) by MTT assay. The results of the studies indicated that the extracts of the different parts of the plant exhibited a concentration-dependent cytotoxic effect as indicated in [Table 3 and Figure 3].

The leaf extract of *A. digitata* displayed a strong cytotoxic effect on *the HeLa* cell line followed by the fruit pulp while the stem bark and roots exhibited the least effects, which were almost four times lower than that of the leaves. Cytotoxic effect was more sustained during the first 12 h, thus there was a slight decline at 24 h and subsequently, the effect was reduced during the last 36 and 48 h, respectively [Figure 4]. Interestingly, the leaves at the highest concentration (250 µg/mL) showed an excellent cytotoxic effect with 91.5, 90.8, 85.0, and 82.4% growth inhibition at 12, 24, 36, and 48 h and $IC_{50}$ values of 99.17, 104.41, 162.23 and 170.02 µg/mL, respectively.

The result of this study showed the chemical constituents of the different parts (fruits pulp, leaves, stem bark, and roots) of *A. digitata* and their effects on *HeLa* cell lines. Different chemical compounds such as palmitic acids, phytol, and *β*-sitosterol among others were identified as the major chemical components in the plant, which varies in the different parts. These findings are in agreement with that of previously reported studies.[22]Recently, fatty acids and steroids have shown beneficial effects on inhibiting tumor development.[23] For instance, phytol found in the leaves of *A. digitata* tends to possess anticancer and

immune-enhancing effects.[24]*β*-sitosterol, a steroid, has also been reported to inhibit cancer cell development.[25]

Characterization of phenolic compounds from the extracts of the different parts of the plant led to the identification of 21 constituents (which vary according to the plant parts), comprising 10 phenolic acid derivatives and 10 flavonoids. This result is in agreement with the findings of previous studies for the leaves, fruits pulp, and shell.[11,24]LC-MS/QTOF analysis indicated that the different plant parts of *A. digitata* predominantly contained phenolic acids and flavonoids as the major constituents that could have a vital role in the anti-cervical activity of the plant; thus, these class of secondary metabolites has been linked to the most pharmacological actions of medicinal plants ranging from anticancer, antioxidant, anti-inflammatory and antimicrobial properties among others.[26]

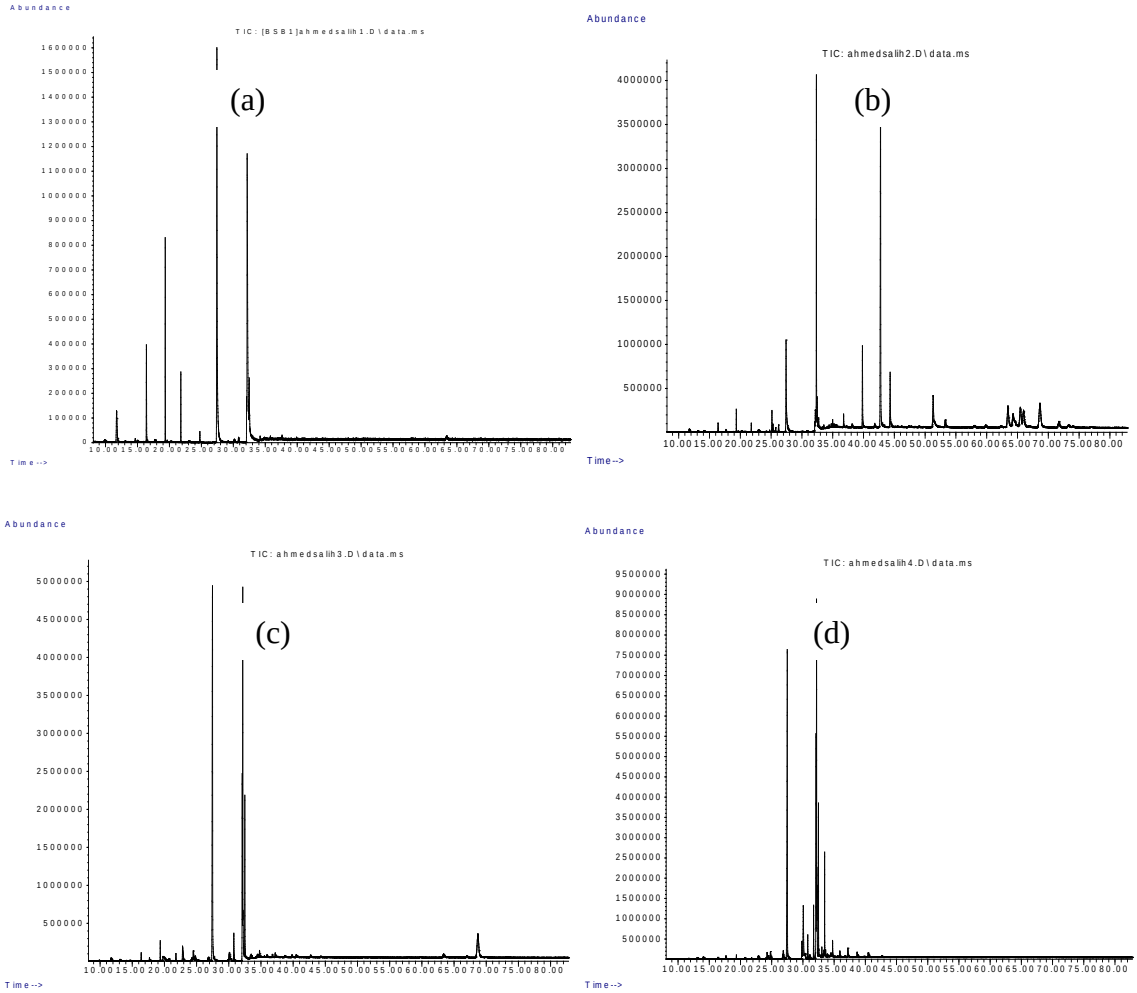


**Figure 1:** Gas chromatogram of (a) fruits pulp, (b) leaves, (c) stem-bark, and (d) roots of *A. digitata* extractsusing GC-MS

**Table 1:** GC-MS analysis of ethanol extracts (fruits pulp, leaves, stem-bark, and roots).

| No. | Compound | Peak area % | | | |
|---|---|---|---|---|---|
| | | **Fruits pulp** | **Leaves** | **Stem-bark** | **Roots** |
| 1 | Palmitic acid | 48.78 | 8.95 | 37.21 | 30.53 |
| 2 | Oleic acid | 40.75 | — | 21.28 | 24.82 |
| 3 | Stearic acid | 6.20 | 2.10 | 8.22 | 6.22 |
| 4 | Linoleic acid | 2.31 | — | 8.80 | 10.38 |
| 5 | Linolenic | — | 1.7 | — | — |
| 6 | Phytol | — | 13.38 | — | — |
| 7 | Octacosane | — | 5.26 | — | — |
| 8 | Squalene | — | 27.06 | — | — |
| 9 | Nonacosane | — | 5.05 | — | |
| 10 | Tetratriacontane | — | 4.38 | — | — |
| 11 | β-Sitosterol | — | 4.98 | — | — |
| 12 | Fridelin | — | 3.60 | — | — |

| | | | | | |
|---|---|---|---|---|---|
| 13 | Amyrin | — | 12.26 | — | — |
| 14 | Germanicol | — | 5.62 | — | — |
| 15 | Lauric acid | — | — | 1.53 | — |
| 16 | Myrisitic acid | — | — | 2.46 | — |
| 17 | Octadecenoic acid | — | — | 1.55 | — |
| 18 | heptadecenoic acid | — | — | 2.11 | 7.29 |
| 19 | Margaric acid | — | — | 2.50 | — |
| 20 | Lupeol | — | — | 8.63 | — |
| 21 | Palmitoleic acid | — | — | — | 1.50 |
| 22 | Nonadecenoic acid | — | — | — | 4.36 |

—: Compound not detected or detected in minor trace < 1%

**Table 2:** LC-MS/QTOF analysis of phenolic compounds in *A. digitata* extracts

| | | Retention time | | | |
|---|---|---|---|---|---|
| **No** | **Compound** | **Fruits pulp** | **Leaves** | **Stembark** | **Roots** |
| 1 | Fumaric acid | 3.06 | 3.06 | 3.06 | 3.06 |
| 2 | Catechin | 5.67 | 5.67 | 5.67 | 5.67 |
| 3 | Gentisic acid | 4.48 | 4.78 | 4.78 | 4.78 |
| 4 | 4-hydroxybenzoic acid | 6.57 | 6.57 | 6.57 | 6.75 |
| 5 | Syringic acid | 6.73 | 6.73 | 6.73 | 6.73 |
| 6 | Vanillic acid | 9.02 | 9.02 | 9.02 | 09.02 |
| 7 | Cinnamic acid | 14.58 | 14.58 | 14.58 | 14.58 |
| 8 | Morin | 14.0 | 14.0 | 14.0 | 14.0 |
| 9 | Diosmin | — | 9.75 | 9.75 | 9.75 |
| 10 | Naringin | — | 11.96 | 11.96 | 11.96 |
| 11 | Protocatechuic acid | — | 12.91 | 12.92 | 12.91 |
| 12 | Chlorogenic acid | | — | 5.76 | — |
| 13 | Rutin | — | 9.20 | 9.20 | — |
| 14 | Scutellarin | — | — | 8.57 | 8.57 |
| 15 | Quercetin-3-*β*-d-glucoside | — | 9.75 | — | — |
| 16 | Sinapic acid | — | — | 18.32 | — |
| 17 | Apigetrin | — | 10.91 | — | — |
| 18 | Neohesperidin | — | — | 10.96 | — |
| 19 | Polydatine | — | — | 9.52 | — |
| 20 | Caffeic acid | 7.46 | — | — | — |
| 21 | Apigenin | — | 0.0075 | — | — |
| 22 | Gallic Acid | 2.34 | 2.34 | 2.34 | 2.34 |

—: Compound not detected

The results of anti-cervical activity indicated that the extracts of the different parts of *A. digitata* exhibited significant and concentration-dependent cytotoxic effects against *HeLa* cell lines at different time intervals, which decreases as the time increases with the leaves showing the highest cytotoxic effect. Thus, the only explanation for this could be related to the fact that the extract reaches its threshold in eliciting its pharmacological effect in the first few hours which decreases with time. These findings might be linked to the presence of flavonoids such as rutin and other phenolic acids in the plant parts, especially the leaves.[27] Several studies have documented the anticancer effects of rutin.[28-31] It appears that no previous study reported the anti-cervical cancer activity of different parts of *A. digitata*. Hence, this novel pharmacological activity suggests that the plant parts, especially the leaves can provide a promising source of cytotoxic compounds against cervical cancer.

## Conclusion

The major chemical constituents of *A. digitata* were found to be fatty acids (such as palmitic acid, oleic acid, phytol, etc), steroids (such as *β*-Sitosterol), and phenolic compounds such as diosmin, catechin, gentisic acid, 4-hydroxybenzoic acid, quercetin-3-*β*-*d*-glucoside, andrutin. *A. digitata* L. has been investigated for anti-cervical cancer activity for the first time. The leaves of the plant appeared to be very promising and can be pursued further for its use as an anti-cervical cancer agent towards drug development. Most of the chemical constituents identified in the different plant parts are common and they have been reported to have good anticancer activity. This study indicated the promising potentials of *A. digitata* leaves as a good source of cytotoxic compounds against

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cervical cancer for future applications in food and pharmaceutical industries.

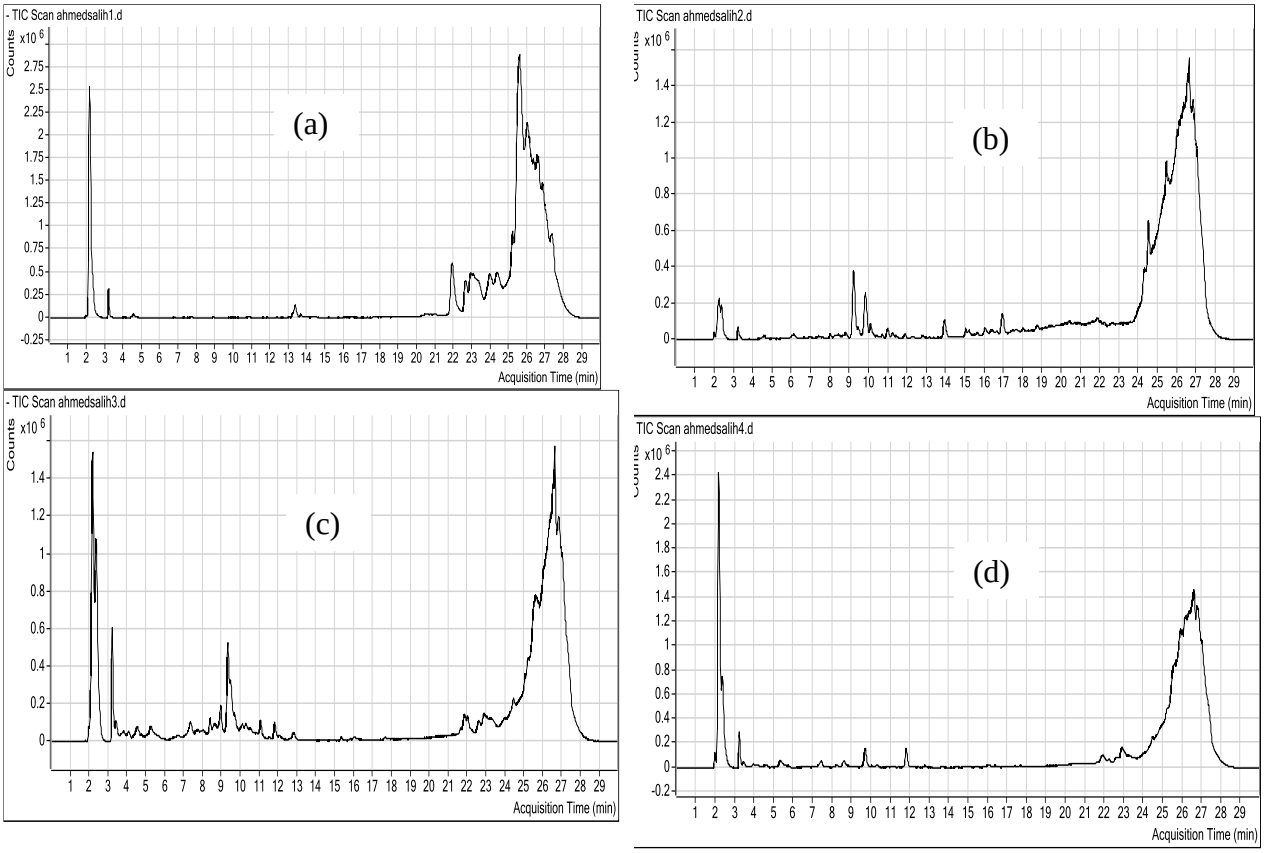


**Figure 2:** LC-MS/QTOF chromatogram of (a) fruits pulp, (b) leaves, (c) stem-bark, and (d) roots of *A. digitata*extracts

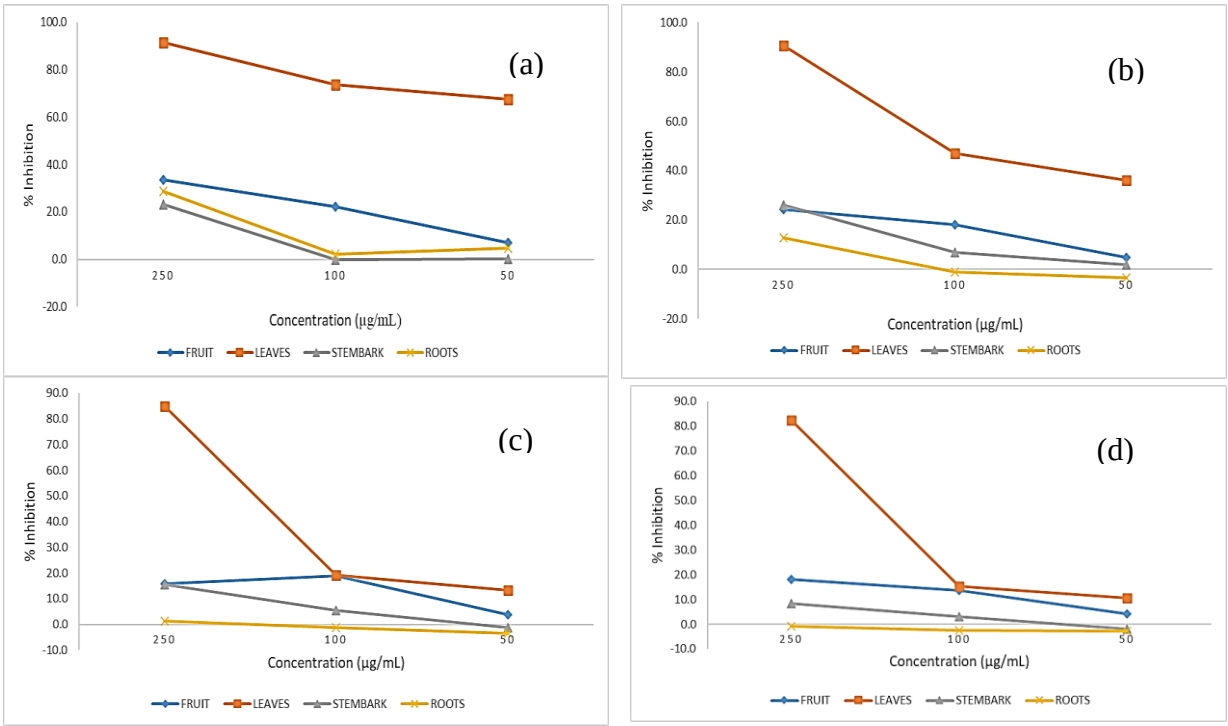


**Figure 3:** % Inhibition of *HeLa* cells after treatment with different extracts (fruits pulp, leaves, stem-bark, and roots) of *A. digitata* at (a) 12, (b) 24, (c) 36, and (d) 48 hours

**Table 3:** $IC_{50}$ values for the cytotoxic effects of the fruit pulp, leaves, stem-bark and roots of *A. digitata*

| | $IC_{50}$ Values (µg/mL) | | | |
|---|---|---|---|---|
| **Plant part** | **12 h** | **24 h** | **36 h** | **48 h** |
| Fruit pulp | 381.21 | 536.45 | 830.50 | 750.44 |
| Leaves | 99.17 | 104.41 | 162.23 | 170.02 |
| Stem-bark | 474.31 | 444.77 | 671.03 | 1099.30 |
| Roots | 421.34 | 700.97 | 2455.02 | 5855.68 |

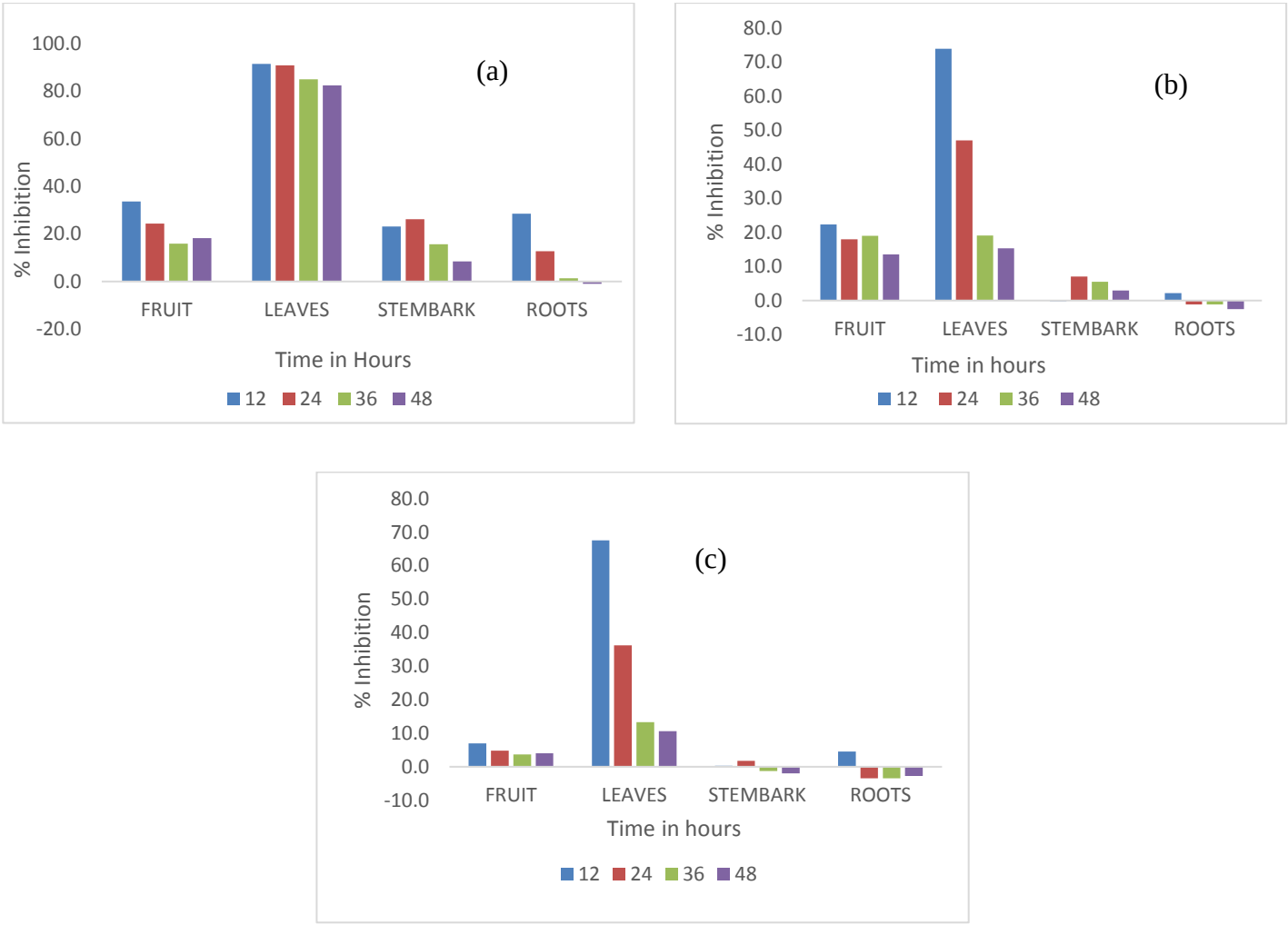


**Figure 4:** Effect of concentration of the different extracts (fruits pulp, leaves, stem-bark, and roots) of *A. digitata* against *HeLa*cells at different time intervals; (a) at 250, (b) at 100, and (c) at 50 µg/mL

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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