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Biological Evaluation of the Aqueous Methanolic Extract and Different Fractions of *Oroxylum indicum* as Cytotoxic, Antimicrobial and Antioxidant Agents

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Abstract: Different biological activities of *Oroxylum indicum* aerial part aqueous methanolic extract and different fractions were assessed. They were subjected to evaluate their cytotoxic, antimicrobial and antioxidant activities as well as evaluating their total phenolics and flavonoids content. The *O. indicum* aerial part ethyl acetate (EtOAc) fraction, showed richness in phenolics and flavonoids content, displaying 153.46 µg GAE/mg extract (total phenolics content) and 158.17 µg RE/mg extract (total flavonoids content) when compared to other extracts and fractions. Therefore, the EtOAc fraction exhibited the strongest antioxidant activity. All fractions showed variable degrees of antibacterial behavior against certain Gram-positive and Gram-negative bacterial strains. On the other hand, only the EtOAc fraction exhibited antifungal activity. Cytotoxicity was examined on two human cancer (MCF-7 and HepG-2) cell lines. However, no significant cytotoxic activity was observed for all extracts except for the *n*-hexane (lipophilic) fraction which showed a minimal activity against both cell lines. A remarkable relationship was found between total phenolics content and biological activity. As the EtOAc fraction showed the highest phenolic and flavonoid contents, this reflects its strongest antioxidant and antibacterial behaviors than other fractions. The *O. indicum* aerial part extracts, specially the EtOAC one offers a valuable source of antioxidant and antibacterial agents with no cytotoxic level. However, several researches are directed recently towards finding natural antioxidants from natural sources. Thus, *O. indicum* can be exploited safely to pharmaceutical industry with a good safety and commercial profile.

Keywords: *Oroxylum indicum*; aqueous methanolic extract; antioxidant; phenolics; flavonoids.

1. INTRODUCTION

Many blooming plants in the Lamiales order are found in the family Bignoniaceae. It's commonly referred to as trumpet vines or bignonias. The majority of Bignoniaceae species are woody, although some are also sub-woody, existing as vines or subshrubs [1]. In three solely herbaceous genera: Toursettia, Argylia, and Incarvillea, there are less herbaceous plants found in high-elevation montane habitats. There are numerous lianas in this family that may ascend using twining, tendrils, or, in rare cases, aerial roots. The Bignoniaceae family comprises around 112-120 genera and more than 725 species [2]. Mostly, it is distributed in tropical regions, with fewer species belong to the temperate regions. Thus, it is widely distributed in northern South America such as Flora of China, Neotropica and Malesiana. On the other hand, it hasn't yet spread to other regions like the Flora of North America and Australia [3].

The genus *Oroxylum* is indigenous to the Indian subcontinent and the foothills of the Himalayas, with smaller populations in Bhutan, Malaysia, and South China [2, 4]. It is represented by only one species called indicum. In the flora of Egypt, *Oroxylum indicum* is

cultivated in the Zoo Botanical Garden, in Giza [5]. *O. indicum*, a small to medium-sized deciduous tree found throughout tropical and subtropical climates, is a member of the Bignoniaceae family and its English name is Indian trumpet flower, however, commonly it is known as "shyonaka" [2, 6].

Because of its healing features, *O. indicum* has been widely used in traditional medicine to treat a wide range of illnesses. Every part of this plant has many medicinal properties such as leaves, fruits, seeds, stem and root bark. The root bark possess astringent and bitter tonic properties, thus are traditionally used in different famous tonic formulations [2]. Bark extracts as its decoction is administered for gastric ulcer treatment while its powder is applied as a paste mouth cancer and skin diseases [7]. Mature fruit extract is used to treat indigestion, cardiac disorders, hemorrhoids, cough and leukoderma [2]. The seeds of *O. indicum* in China is also employed for treatment of liver and stomach disorders while, in Malaysia employed for treating toothache, fever, cholera and splenomegaly [2, 8].

Numerous phytoconstituents, including flavonoids, phenolics, anthraquinone and sterols, have been extracted from *O. indicum*, according to a thorough review of the literature [8]. Meanwhile, baicalein, chrysin and apigenin are flavonoids isolated from the seeds. Also, new flavonoids that have gastroprotective activity have been isolated from the stem bark [2].

Oroxylum indicum offers an opportunity for bio prospection as different parts of this plant have an effective use in Ayurvedic preparations and herbal tea formulations. By assessing the cytotoxicity, antimicrobial, and antioxidant activities of *O. indicum*, this study aims to give a thorough analysis of the plant's potential therapeutic properties. It also hopes to shed light on the plant's potential uses in the pharmaceutical sciences and advance our knowledge of its medicinal value.

2. METHODS

2.1. Reagents and chemicals

HepG-2 (a cell line for hepatocellular cancer) and MCF-7 (a cell line for breast carcinoma) were obtained from the VACSERA Tissue Culture Unit in Cairo, Egypt. The MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, DMSO (dimethyl sulfoxide), ascorbic acid, trypan blue, crystal violet, and DPPH (2,2-Diphenyl-1-picrylhydrazyl) was supplied by Sigma Chemical Co. (St. Louis, Mo., USA). Other chemicals were acquired from El-Nasr Pharmaceutical Company in Cairo, Egypt, including *n*-hexane, *n*-butanol, ethyl acetate (EtOAc), and methanol (MeOH).

2.2. Plant material

In May 2021, the *O. indicum* aerial part was collected from the Zoo Botanical Garden in Giza, Egypt. Abdel-Halim Abdel-Magly who is a professor in the Horticulture Research Institute's Flora and Phyto-taxonomy Research Unit in Giza, Egypt, identified and authenticated the plant. The Pharmacognosy Department at Faculty of Pharmacy (Girls), Al-Azhar University in Cairo is home to the herbarium of specimens.

2.3. The plant extract and fractions preparation

The aerial part was first allowed to dry at ambient temperature before being crushed into a fine powder for the purposes of the current study. By soaking 564 g of powdered aerial part in methanol (3 x 3 L), filtering, and evaporating under decreased pressure until dryness was achieved, a 70% total methanolic extract (246 g) was extracted. Then, by using *n*-hexane (non-polar solvent), a 0.28 g of dried lipophilic fraction was obtained. Also, the dried residue was extracted with ethyl acetate (EtOAc) to yield 35.37 g and with *n*-butanol to yield 14.77 g. Finally, 124.59 g of the aqueous extract was remained.

2.4. Spectrophotometric methods used in the estimation of total phenolics and flavonoids content in the aqueous methanolic extract and different fractions

The total phenolic content was determined according to the procedure adopted by **Attard *et al.*, 2013** using Folin Ciocalteu colorimetric method [9]. A stock solution of gallic acid (2 mg/mL) in methanol was prepared, with the following dilutions: 1000, 750, 500, 375, and 250 µg/mL. Thus, a calibration curve for gallic acid was obtained and the absorbance was determined at 765 nm (**Figure 1**). The OIB (*n*-hexane =lipophilic) and OIC (ethyl acetate =EtOAc) fractions were prepared in ethanol with a 5 mg/mL concentration while, a concentration of 10 mg/mL of the OIA (total methanolic extract), OIE (aqueous extract) and OID fraction (*n*-butanol fraction) was prepared in ethanol. In brief, the procedure involved mixing 10 µL of sample/standard with 100 µL of Folin-Ciocalteu reagent (Diluted 1: 10) in a 96-well microplate. Then, 80 µL of 1M Na_2CO_3 was added and incubated at room temperature (25 °C) for 20 min in the dark. At the end of incubation time, the resulting blue complex color was measured at 630 nm. The total polyphenols content of the extract expressed was mg of Gallic Acid Equivalent (GAE) to plant dry weight.

On the other hand, the total flavonoids content was determined using the aluminum chloride method with small modifications to be performed in

microplates [10, 11]. A stock solution of Rutin as a standard was prepared at 2000 µg/mL in methanol with preparing the following dilutions: 1000, 500, 250, 125, and 62.5 µg/mL. A concentration of 5 mg/mL of the OIB and OIC fractions was prepared in ethanol while, a 10 mg/mL concentration of the OIA, OIE extracts, and OID fraction was prepared in ethanol. In brief, in a 96-well microplate, a 15 µL of sample/standard was placed, then followed by 175 µL of methanol and 30 µL of 1.25 % $AlCl_3$. Finally, a 30 µL of 0.125 M $C_2H_3NaO_2$ was added and incubated for 5 min. After incubation, the resulting yellow color was measured at 420 nm (**Figure 2**).

In both methods, data are represented as means ± SD (Standard deviation) and a microplate reader FluoStar Omega was used to record the results.

2.5. Biological study

2.5.1. In vitro cytotoxic activity

The cytotoxic effects of *O. indicum* aqueous methanolic extract and fractions were evaluated using the MTT assay. Using 100 µL growth media, cells were exponentially seeded in flat-bottomed 96-well microtiter plates (Falcon, NJ, USA) at a density of 10,000 cells per well. The cells were then left to adhere for a full day. Using a multichannel pipette, the tested material was then introduced in two-fold serial dilutions to confluent cell monolayers. Cells that were left untreated, treated with DMSO (solvent control), and treated with a recognized cytotoxic agent (positive control) were all used as controls. The experiment was shown to not be disturbed by the lowest dose of 0.1% DMSO in the wells that was added [12]. Following the treatment phase, each well received 10 µL of MTT reagent (5 mg/mL in PBS), and the plates were incubated at 37°C for 4 hours. After the completion of the incubation time, a 1% of (crystal violet solution) was added for each well at least 30 minutes.

The plates were cleaned with tap water after the stain elimination to eliminate any remaining residue. A microplate reader was used to measure the absorbance at 490 nm. Every experiment was run in triplicate. This equation was used to calculate the cell viability percent:

Percentage of cell viability = [ODt / ODc] x 100%

Where, ODt represents the density of the mean optical of wells treated with the tested sample and ODc is the untreated cells mean optical density.

GraphPad Prism software was used for the IC_{50} value calculation from dose-response curves at each concentration and to calculate cell viability as a percentage relative to the control group [12]. The results indicated a dose-dependent cytotoxic effect of *O. indicum* aerial part aqueous methanolic extract and fractions on the tested cell lines, demonstrating its potential as a source of cytotoxic agents.

2.5.2. Anti-microbial assay

Using the standard agar well diffusion method, the antibacterial activity of the aerial part whole aqueous methanolic extract and different fractions was assessed against standard strains of Gram-positive and Gram-negative bacteria as well as various fungal strains [13]. By following the Clinical Laboratory Standards of the National Committee, a concentration of 10 mg/mL was tested against various microorganisms. Measuring the presence of the inhibitory zones following a 24-hour incubation period allowed for the establishment of the antibacterial and antifungal activities with positive results.

2.5.2.1. Microorganisms

Aspergillus fumigatus (RCMB 002008) and *Candida albicans* (RCMB 005003 (1) ATCC 10231) are the fungi that were utilized. *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (RCMB 015 (1) NRRL B-543) are the Gram-positive bacterial strains utilized, whereas *Escherichia coli* ATCC 25922 and *Proteus vulgaris* RCMB 004 (1) ATCC 13315 are the Gram-negative bacterial strains used.

2.5.2.2. Anti-bacterial assay

Luria Bertani agar plates were coated with bacterial liquid culture (100–200 µL) during an exponential growth phase, for a test including both Gram-positive and negative bacteria. Disc paper (5 mm diameter, Oxoid Ltd.) was loaded with 10–20 µL of tested samples right away, resulting in final disc with loading concentrations from 250 to 500 µg for sample extracts. Together with discs containing solvent blanks, the impregnated discs were put on the surface of Luria Bertani agar that had previously been seeded with the chosen test organisms. The inoculated plates are incubated at the appropriate temperature (typically 37°C for bacteria) for a specified period (commonly 24-48 hours). This allows the test microorganism to grow and the test substances to diffuse through the agar. After incubation, the plates are examined for zones of inhibition which is the clear areas around the wells where microbial growth has been inhibited by the diffused test substances. The diameter of these zones is measured using a calibrated ruler. The diameter in mm of the inhibition zones is recorded and compared with control results to evaluate the antimicrobial activity of the test substances. Greater potency against the test microorganism is indicated by larger zones. As indicated in **Table 4**, a positive control of 4 µg/mL Gentamycin was employed.

2.5.2.3. Anti-fungal assay

The surface of the potato dextrose agar medium was covered with 100 mL of the fungal spore suspension. Disc paper (5 mm diameter, Oxoid Ltd.) was loaded with 10–20 µL of the tested samples right away, resulting in final disc with loading concentrations from 250 to 500 µg for the tested extracts. The potato dextrose agar medium's surface was coated with impregnated discs. Following many days of room temperature incubation, the fungal cultures were examined for growth inhibition around the discs. Next, the outcome was contrasted with a positive control of 100 µg/mL ketoconazole, as indicated in **Table 4**.

2.5.3. Anti-oxidant assay

The (DPPH) radical scavenging experiment was used to examine the anti-oxidant activity of *O. indicum* aerial part aqueous methanolic extract and different fractions [14]. This experiment was performed in the Regional Center for Mycology and Biotechnology at Al-Azhar University, Cairo, Egypt. Freshly prepared DPPH radical methanol solution was used, protected from light and stored at 10 °C. A 10-1280 µg/mL was prepared in a methanol solution of the tested samples. Three milliliters of DPPH solution were mixed with a methanol solution (40 uL aliquot). Next, utilizing a UV-visible spectrophotometer (Milton Roy, Spectronic 1201), the absorbance was promptly measured. The drop in absorbance was constantly measured at 515 nm, with data being collected every minute. To create a standard curve, ascorbic acid was utilized as the standard antioxidant. Measurements were also obtained from the DPPH radical (control) and the absorbance of ascorbic acid (reference substance).

Each calculation was performed three times, then averaged. The percentage of inhibition was determined using the following method [15]:

$$\% \text{ inhibition} = 1 - \frac{A_C - A_T}{A_C} \times 100$$

Where, A_C represents the control absorbance at t = 0 min and A_T is the sample absorbance + DPPH (at t = 16 min).

The results described an increase in DPPH radical scavenging activity with increasing concentrations of *O. indicum* total methanolic extract and fractions. The dose-response curve was used to calculate the value of IC_{50}, which refers to the concentration detected to inhibit 50% of the DPPH radical and highlights the extracts under investigations having strong antioxidant potential.

3. RESULTS

3.1. Estimation of total phenolics and total flavonoids content in the aqueous methanolic extract and different fractions

The following Table 1 illustrates a comparative evaluation of the total phenolics content between the aqueous methanolic extract and different fractions in which, the phenolics content in the tested samples is expressed as Gallic Acid Equivalent (GAE) (Figure 1).

Furthermore, a comparative determination of the total flavonoids content between the same fractions is illustrated in the following Table 2. The total flavonoids content in the tested samples is expressed as Rutin Equivalent (R E) (Figure 2).

3.2. Biological Study

3.2.1. Cytotoxic activity

The cytotoxic activity of *O. indicum* aerial part aqueous methanolic extract and different fractions was assessed on two human cancer cell lines: MCF-7, which is used for breast cancer, and HepG-2, which is used for hepatocellular carcinoma as shown in Figures 3 & 4. The IC_{50} profile for both cell lines was described in Table 3.

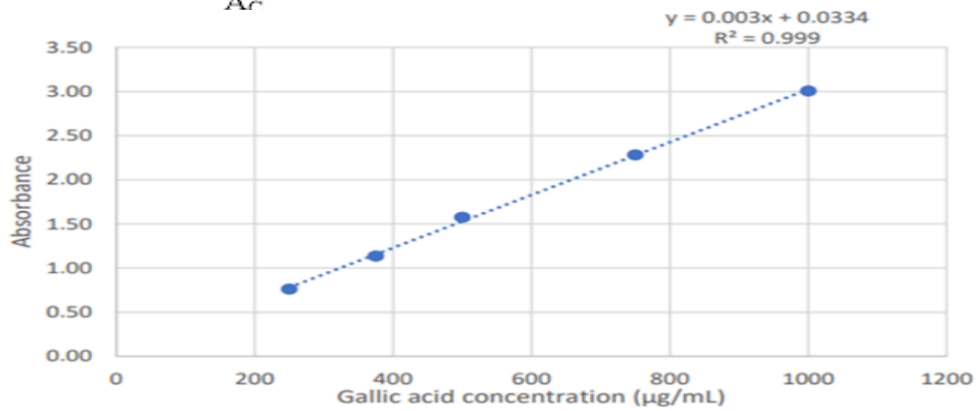

Figure 1. Calibration curve for Gallic acid.

Table 1. Results of comparative evaluation of the total polyphenolics content in *Oroxylum indicum* aerial part.

Sample ID	Average reading at 630 nm	Substitution in calibration curve equation (µg GA/mg sample)	Total phenolics content (µg gallic acid per 1mg sample)	Standard deviation
OIA*	0.48	150.03	16.38	1.16
OIB	1.32	430.03	88.75	9.26
OIC	2.10	689.53	153.46	13.11
OID	0.91	291.78	29.18	0.80
OIE	NA	NA	NA	NA

* The absorbance of the sample OIA was below the calibration curve. The maximum soluble concentration for OIA and OIE was 10 mg/ml in ethanol. (OIA) represents (total methanolic extract), (OIB) lipophilic fraction, (OIC) EtOAc fraction, (OID) *n*-butanol fraction, and (OIE) represents the remaining aqueous extract. NA represents no activity.

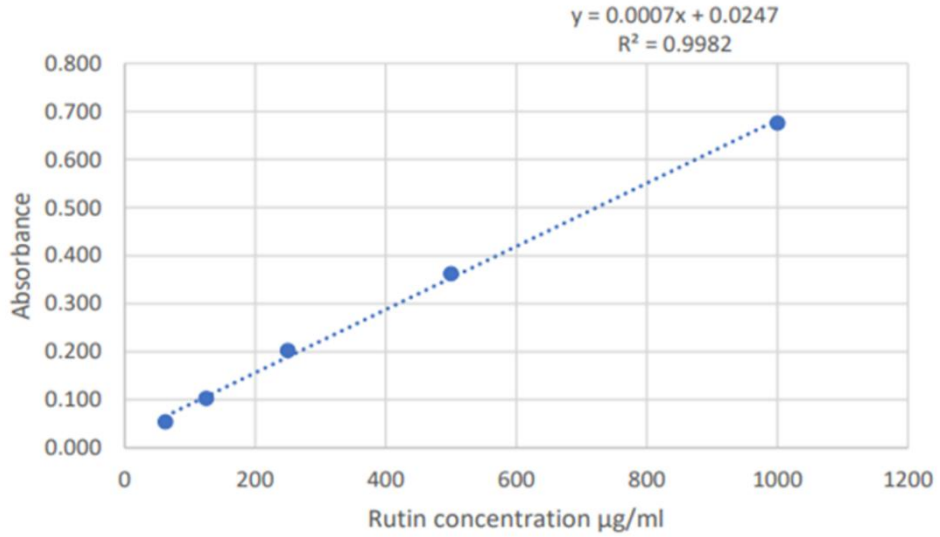

Figure 2. Calibration curve for Rutin.

Table 2. Results of comparative determination of the total flavonoids content in O. indicum aerial part.

Sample ID	Average reading at 420 nm	Substitution in calibration curve equation (µg RE/mg sample)	Total phenolics content (µg RE per 1mg sample)	Standard deviation
OIA	0.12	141.86	14.19	0.38
OIB	0.16	186.62	30.80	3.03
OIC	0.32	415.43	158.17	3.23
OID*	0.04	18.05	1.80	0.66
OIE	NA	NA	NA	NA

* The absorbance of the sample OID was below the calibration curve. The maximum soluble concentration for OID and OIE was 10 mg/ml in ethanol. (OIA) represents (total methanolic extract), (OIB) lipophilic fraction, (OIC) EtOAc fraction, (OID) *n*-butanol fraction, and (OIE) represents the remaining aqueous extract. NA represents no activity.

3.2.2. Antimicrobial activity

Also, they were evaluated for their antimicrobial activity against standard bacterial and fungal strains as described in Table 4 that lists the growth inhibition zones average diameters**.**

3.2.3. Antioxidant activity

Moreover, the *O. indicum* fractions were assessed for possible antioxidant activity. It was established what concentration (IC_{50}) was used for DPPH radical inhibition by 50% as shown in Figure 5 and tabulated in Table 5.

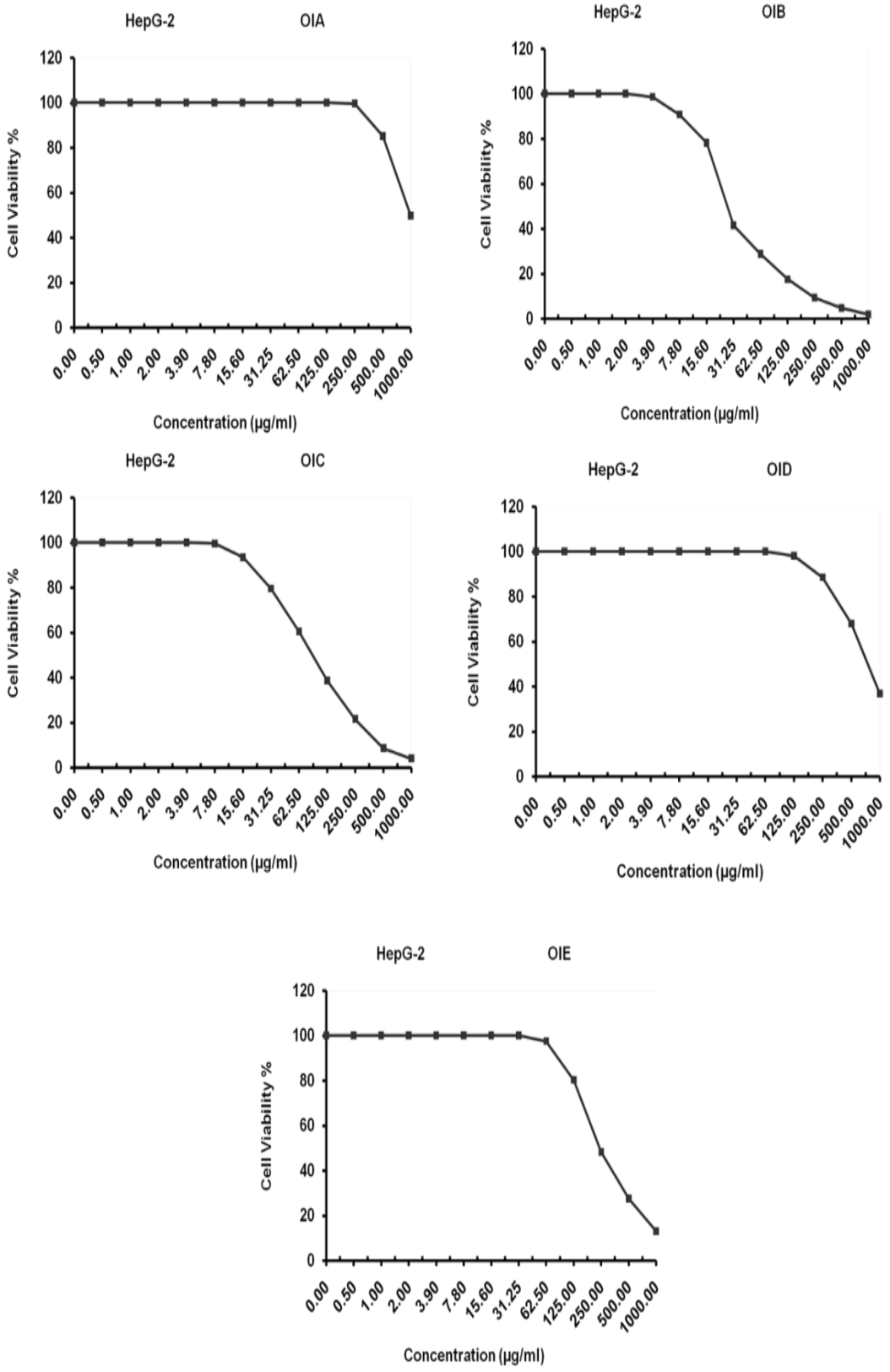

Figure 1. Cytotoxic activity of (OIA) total methanolic extract, (OIB) lipophilic fraction, (OIC) EtOAc fraction, (OID) n-butanol fraction and (OIE) aqueous extract of O. indicum aerial part on HepG-2 cell lines.

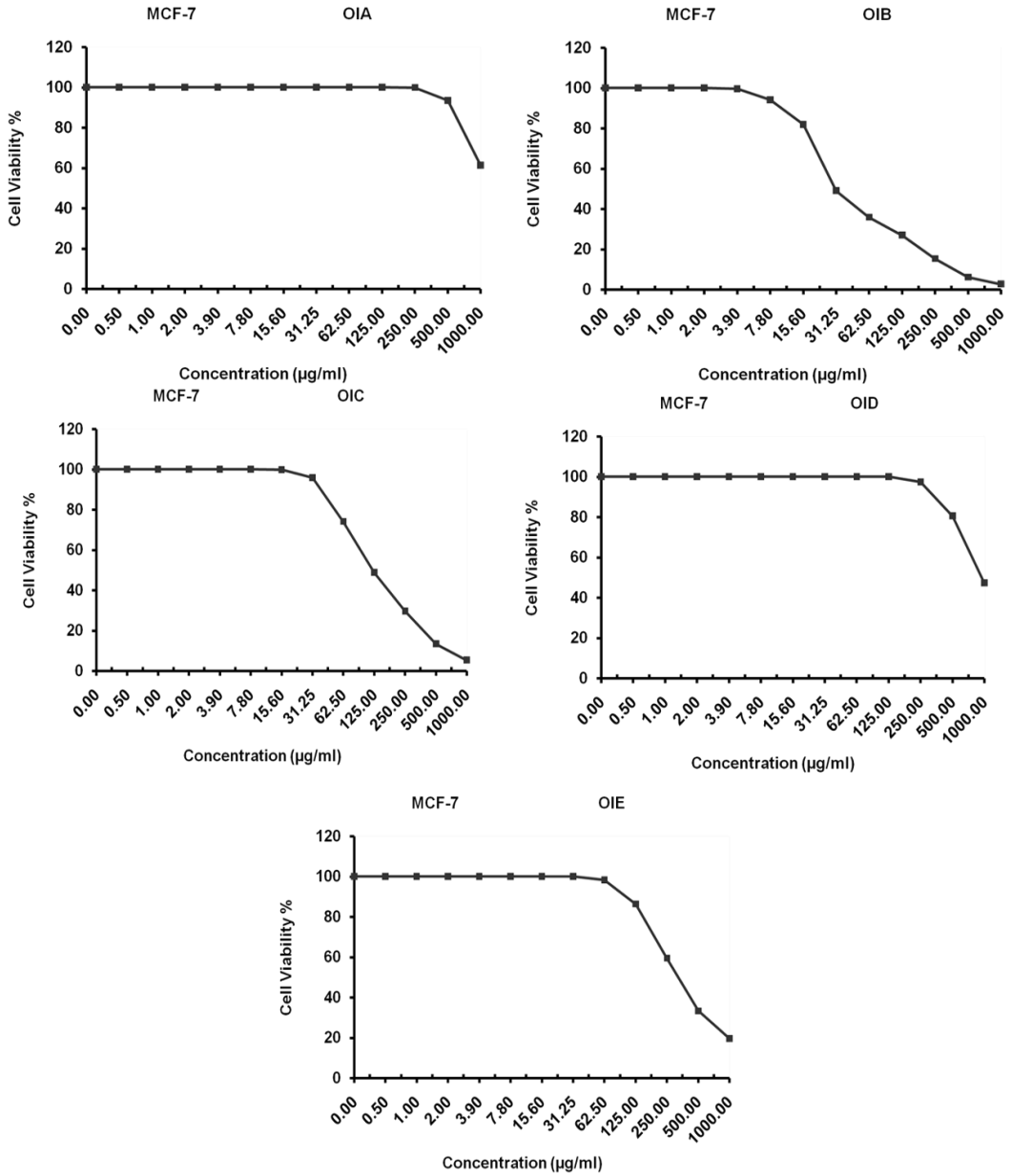

Figure 4. Cytotoxic activity of (OIA) total methanolic extract, (OIB) lipophilic fraction, (OIC) EtOAc fraction, (OID) n-butanol fraction and (OIE) aqueous extract of O. indicum aerial part on MCF-7 cell lines.

Table 3. IC_{50} values of *O. indicum* aerial part aqueous methanolic extract and different fractions using MTT assay.

Plant extract/fraction	IC_{50} Values (µg/mL)	
	HepG-2 Cell line	MCF-7 Cell line
OIA	996.04 ± 26.71 µg/ml	Weak activity
OIB	27.53 ± 1.74 µg/ml	30.73 ± 1.93 µg/ml
OIC	92.30 ± 3.14 µg/ml	121.93 ± 2.67 µg/ml
OID	787.56 ± 14.23 µg/ml	958.01 ± 16.98 µg/ml
OIE	242.82± 3.96 µg/ml	339.96 ± 6.72 µg/ml
Standard (Vinblastine Sulfate)	3.68 ±0.17 µg/ml	3.78 ± 0.54 µg/ml

(OIA) represents (total methanolic extract), (OIB) lipophilic fraction, (OIC) EtOAc fraction, (OID) *n*-butanol fraction, and (OIE) represents the remaining aqueous extract.

Table 4. The aqueous methanolic extract and different fractions mean zone of inhibition of the aerial part, calculated in millimeters. [a]

Tested microorganisms	OIA	OIB	OIC	OID	OIE	Control
FUNGI						*Ketoconazole*
***Aspergillus fumigatus* (RCMB 002008)**	NA	NA	12	NA	NA	17
***Candida albicans* RCMB 005003 (1) ATCC 10231**	NA	NA	10	NA	NA	20
Gram Positive Bacteria						*Gentamycin*
***Staphylococcus aureus* ATCC 25923**	16	20	19	13	11	24
***Bacillus subtilis* RCMB 015 (1) NRRL B-543**	10	14	15	10	9	26
Gram Negatvie Bacteria						*Gentamycin*
***Escherichia coli* ATCC 25922**	15	NA	18	NA	NA	30
***Proteus vulgaris* RCMB 004 (1) ATCC 13315**	12	13	19	11	10	25

[a] Positive management of fungus 100 µg/mL of ketoconazole. Positive control for Gentamycin is 4 µg/mL. (OIA) represents (total methanolic extract), (OIB) lipophilic fraction, (OIC) EtOAc fraction, (OID) *n*-butanol fraction, and (OIE) represents the remaining aqueous extract. NA represents no activity. At a concentration of 10 mg/mL, the sample was analyzed. The diameters of the growth inhibition zones are given in average in millimeters.

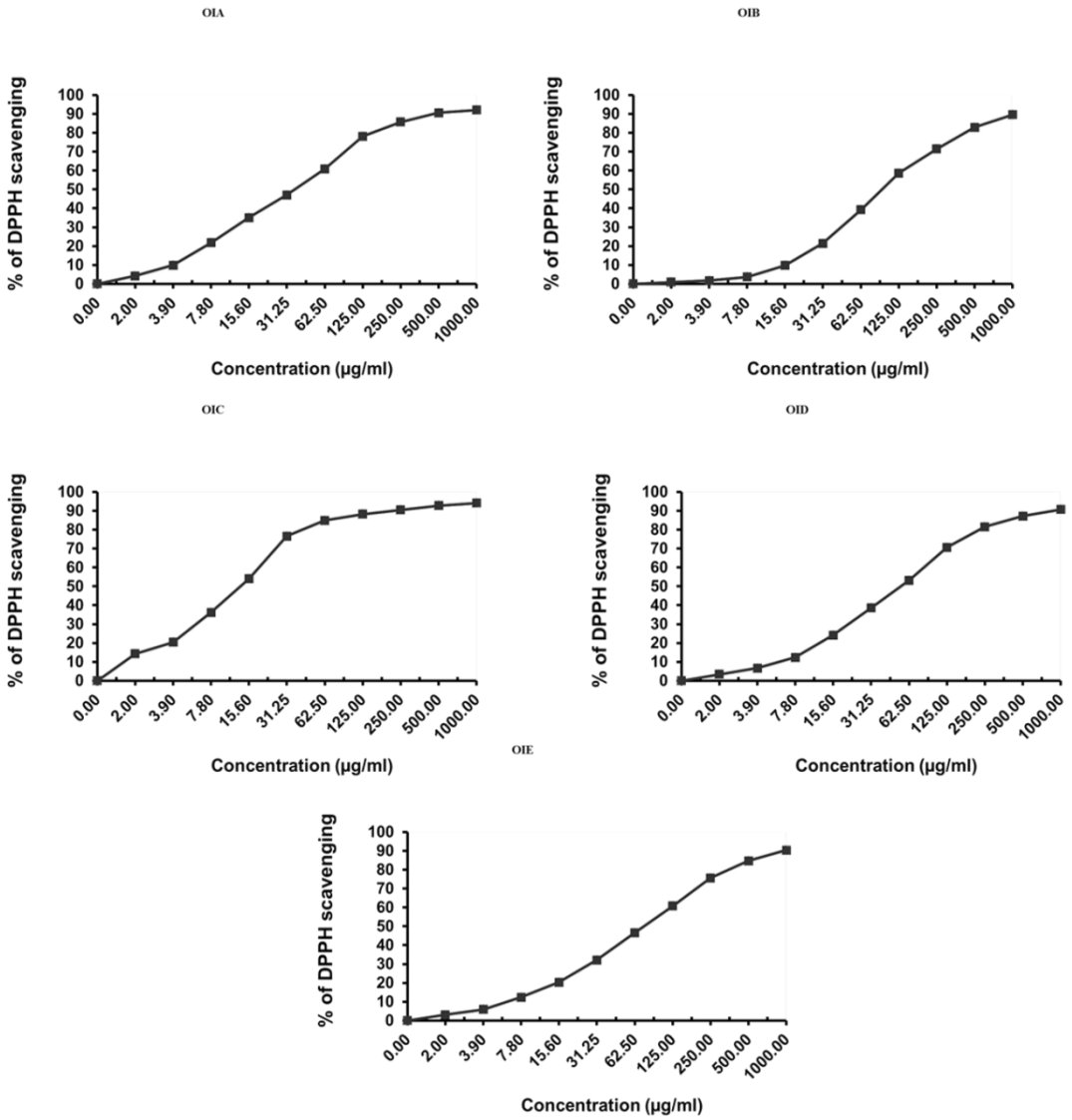

Figure 2. Antioxidant activity of (OIA) total methanolic extract, (OIB) lipophilic fraction, (OIC) EtOAc fraction, (OID) *n*-butanol fraction and (OIE) aqueous extract of *O. indicum* aerial part.

Table 5. IC_{50} values of *O. indicum* aerial part aqueous methanolic extract and different fractions using DPPH assay.

Plant extract/fraction	IC_{50} Values (µg/mL)
OIA	73.80 ± 2.09 µg/mL
OIB	97.09 ± 4.71 µg/mL
OIC	13.84± 0.59 µg/mL
OID	55.67±2.74 µg/mL
OIE	77.35 ±3.96 µg/mL
Standard (Ascorbic acid)	**14.2 µg/mL**

(OIA) represents (total methanolic extract), (OIB) lipophilic fraction, (OIC) EtOAc fraction, (OID) *n*-butanol fraction, and (OIE) represents the remaining aqueous extract.

4. DISCUSSION

Oroxylum indicum (Bignoniaceae), a traditional Indian herbal medicine, possesses awide range of biological activities including antioxidant, anticancer, antibacterial, and anti-inflammatory. Therefore, this study was designed in order to guide and evaluate the practical application of *O. indicum* various extracts as cytotoxic, antimicrobial and antioxidant agents in the pharmaceutical, food, and cosmetic industries. Meanwhile, the extracts possessing an IC_{50} value against the tested cancer cell lines < 20 µg/mL according to the American National Cancer Institute (NCI), are considered to be cytotoxic [16, 17]. Thus, it becomes a benchmark for significance of an anticancer agent of crude extract and as applied on the aqueous methanolic extract and fractions cytotoxicity results, the OIB fraction revealed considerable cytotoxic activity and can be used as a promising cytotoxic agent against HepG-2 and MCF-7 human cancer cell lines. The OIA extract exhibited a weak cytotoxic activity as long as the OIC fraction had a mild cytotoxic effect on both cell lines. However, OID and OIE fractions exhibited non-significant inhibitory activity against the two cell lines. It was reported that *O. indicum* non-polar extracts can effectively induce apoptosis by targeting Estrogen Receptor negative breast tumor cells without causing harm to normal cells by cancer-specific cytotoxicity manner. Also, at a concentration of 0.05%, the ethanolic fraction exhibited cytotoxic activity against HepG-2 cell line [2, 18].

The terms commonly used to evaluate the results of the antimicrobial assay are that, an inhibition zone of < 9 mm was deemed inactive, 9–12 mm was deemed partially active, 13–18 mm was deemed active, and more than 18 mm was deemed very active [19, 20]. Thus, the OIA fraction exhibited partial activity against *Bacillus subtilis,* and *Proteus vulgaris* and was active against *Escherichia coli* and *Staphylococcus aureus*. On the other hand, the OIB and the OIC were considered very active against *S. aureus* and active against *B. subtilis*; however, no activity was observed against *E. coli* but active against *P. vulgaris* for OIB fraction, while the OIC fraction was considered very active against both gram-negative bacterial strains. In addition, the OID fraction exhibited activity against *S. aureus* and partial activity against *B. subtilis*. Unlike, the OIE fraction showed partial activity against both gram-positive bacterial strains. Also, no activity was observed against *E. coli* for both fractions (OID & OIE) while, they exhibited partial activity against *P. vulgaris*. No antifungal activity was observed for all the total extract and fractions except for the OIC fraction that exhibited partial antifungal activity. It was evident from published research that the petroleum ether and ethyl acetate extracts had strong antibacterial and antifungal action in antibacterial and antifungal assays [21].

The decision of a compound or extract to have a strong antioxidant activity or not is based on what was reported by Molyneux *et al.,* [22] that is "The value of IC_{50} decreases as antioxidant activity increases". Therefore, the OIC fraction exhibited the strongest antioxidant activity when compared with other fractions.

Mainly, this significant antioxidant behavior of the OIC fraction of *O. indicum* aerial part was expected and strongly related to its high content of phenolics and flavonoids as calculated using spectrophotometric methods [23, 24]. It contains the highest phenolic content (153.46 µg/mg gallic acid equivalent) followed by the OIB fraction that contains 88.75 µg/mg gallic acid equivalent. Moreover, the OIC fraction owns the highest flavonoid content (158.17 µg/mg Rutin equivalent) while, the OIB fraction contains 30.80 µg/mg which is considered adequate content compared to that existing in the OIC fraction. In a previous study published by Mishra *et al.,* the leaves extracts revealed containing polyphenolic compounds with high concentrations (124.7 ± 4.36 µg/mg pyro-catechol equivalents) [25].

5. CONCLUSIONS

The results validate and demonstrate the application of *Oroxylum indicum* in traditional remedies for various infections. The lipophilic fraction showed minimal cytotoxic activity on both human cell lines (HepG-2 and MCF-7), when compared with other fractions. A significant antibacterial activity was observed for all the aqueous methanolic extract and fractions. No antifungal activity was observed for all except for the EtOAc fraction. It also exhibited a very significant and strong antioxidant activity because of its high phenolic (Gallic acid equivalent) and flavonoid contents (Rutin equivalent). Meanwhile, the high content of antioxidants in the aerial part supports the use of *O. indicum* in medicinal and Ayurvedic preparations. Given the importance of the pharmaceutical sector, the aerial part of *O. indicum* can be selected as a powerful source of antioxidant chemicals, which bolster the plant's important medicinal potential and can be applied to a range of pharmaceutical applications.

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Author Contribution: Sara S. Kotb: Plant material collection and processing, data analysis and writing the manuscript. **Marwa S. Abubakr:** Supervision, revising the biological data analysis and revising the manuscript. **Ahmed H. Desouky:** Supervision, Data Analysis and revising the manuscript. **Abdelsalam I. Mohamed:** Supervision and revising the manuscript.

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