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In vitro Antileukemic Activity of Extracts of Some Medicinal Plants from Upper Egypt in Human Chronic Leukemia K562 Cell Line

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ABSTRACT

Leukemia is the fourth most common cancer in Egypt, with higher incidence rates in Upper Egypt. Because there are several side effects associated with the use of drugs to treat leukemia, it is crucial to find more effective, safe, and cost-effective alternative treatment options. The present study was conducted to investigate the antileukemic potentials of some medicinal plants which are cultivated and used ethnomedicinally in Upper Egypt, where chronic myeloid leukemia (CML) is prevalent. Fifty-six different medicinal plants were extracted with 75% ethanol, and their hydro-ethanol extracts were tested against the human chronic leukemia K562 cell line at a concentration of 10 mg/mL using the trypan blue exclusion assay. The percentage of inhibition was determined for each extract. The results showed that *Sesbania sesban* L. Merr leaves, *Curcuma aromatica* Salisb. roots, *Spinacia oleracea* L. leaves, *Quercus infectoria* gall, and *Thymus vulgaris* L. leaves were the most active plant extracts against the leukemia K562 cell line, with percentage inhibition values of 100, 90.2, 88.9, 87, and 85.2%, respectively, compared to Taxol which had a value of 90.7%. The findings of this study revealed that the flora of Upper Egypt is a valuable source of plants, rich in antileukemic phytochemicals.

Keywords: Antileukemic, Chronic myeloid leukemia, Medicinal plants, Screening, Upper Egypt.

Introduction

Leukemia is ranked as the fourth most prevalent cancer in Egypt, accounting for about 7.2% of all the reported cancer cases, at all ages, and in both genders.[1] According to the National Population-Based Cancer Registry Program, the incidence rates of myeloid leukemia in Upper Egypt, "areas from Aswan, downriver to the area of El-Ayait",[2] were higher than in Lower Egypt (3.36-3.7% and 0.63-0.65%, respectively).[3] Increasing age, diabetes mellitus, hepatitis C virus, obesity, exposure to agricultural chemicals, and exposure to electromagnetic fields were all linked to an increase in the incidence of hematological malignancies in Upper Egypt.[4] Chronic myeloid leukemia (CML) is a myeloproliferative cancer marked by uncontrolled myeloid cell divisions in the bone marrow.[5] The Philadelphia (Ph) chromosome (Bcr-Abl gene) is a chromosomal irregularity that results from a reciprocal translocation between the Abl gene on chromosome 9 and the Bcr gene on chromosome 22 in pluripotent hematopoietic stem cells.[6,7] The Bcr-Abl oncogene encodes a constitutively active tyrosine kinase enzyme that activates multiple proliferative signaling pathways within cells.[8] Around 57% of Egyptian CML patients were Ph-positive.[9] Several treatment strategies are currently being used in Egypt to treat CML patients. These strategies include using hydroxyurea in 50.6% of patients, Gleevec (imatinib, a tyrosine kinase inhibitor) in 37.2% of patients, and interferon-alpha in 12.2% of patients.[9] According to the reports of complete hematologic responses (CHRs) of CML Egyptian patients to the current medication, the percentage of complete hematologic and cytogenetic responses were 67.2-71.6, 31.9-34.1, and 6-18.7%, respectively, of patients who received Gleevec, hydroxyurea, and a combination of Gleevec and hydroxyurea. According to reports, 21.7% of patients had disease progression.[9] Although, hematopoietic stem cell transplantation (HSCT) is considered an effective therapy, it is not always appropriate due to a lack of suitable donors.

The increased disease progression and response failure in those treated with Gleevec were attributed to treatment interruption due to the high cost of Gleevec therapy, as well as the development of adverse effects.[9] Interrupted treatment results in the loss of acquired therapeutic responses and, in some cases, disease progression.[9-11] Furthermore, resistance to imatinib treatment has been reported in several studies due to a variety of molecular mechanisms, including amplification and overexpression of the Bcr/Abl gene,[12] or overexpression of the MDR1 gene.[13] Recent studies revealed that imatinib resistance in Egyptian CML patients was caused by an association of MDR1 gene polymorphism (G2677T),[14] T315I mutation, which was reported in 24.4% of CML patients in Egypt.[15] Furthermore, low miR-30a expression and high Beclin-1 expression have been linked to resistance to imatinib therapy in Egyptian CML patients.[16] Given the prevalence of diseases among Egyptians, the scarcity of suitable donors for HSCT, the high cost of currently used tyrosine kinase inhibitors (TKIs), the low efficacy of other medications such as hydroxyurea and interferon-alpha, and TKI resistance, it has become vital to search for more effective, safe, and cost-efficient treatments. For several medicinal plants and their isolated secondary metabolites against a CML cell line (K562), the value of natural products in the arsenal of CML therapies has been widely described.[17] Several natural compounds are currently being tested in clinical trials for the treatment of CML, such as Homoharringtonine, in Phase II trials, 17-AAG (analog of glendamycin–polyketide) in Phase I clinical trials, and Paclitaxelin Phase I/II trials.[17] According to the Information and Decision Support Center in Egypt,[18] 23% of Egyptians use medicinal plants to treat their ailments. There are several methods for selecting plants as drug discovery candidates, including random selection followed by chemical or biological screening, ethnomedical use of plants, and

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biologic activity reports.[19,20] The aim of the current research was to investigate the antileukemic activity of medicinal plants cultivated, or utilized by Egyptians in Upper Egypt, where CML is the most common type of leukemia.

Materials and Methods

Sources of plant material

The fifty-six plants used in this study (Tables 1-4) were collected from Beni-Suef and Fayoum governorates during the period from September to December 2017, and some were obtained from herbal vendors and shops. The collected plants belong to thirty-one plant families, including the Asteraceae, Chenopodiaceae, Fabaceae, Lamiaceae, and Poaceae as the major families. The plants were authenticated by Prof. Dr. Lotfy Boulus, Prof. Dr. Mohammed El Gibali, (Faculty of Science, Cairo University), Dr. Mahmoud Omar (Plant Taxonomy Department, Faculty of Science, Beni-Suef University), and Mrs. Therese Labib, (Botanical Specialist and Consultant at Orman and Qubba Botanical Gardens, Giza, Egypt). The voucher specimens (FUPD-1 to FUPD-56) were kept at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Fayoum University, Egypt.

Table 1: Selected medicinal plants from Upper Egypt for biological screening against leukemia K562 cell line

No.	Latin name	Common name	Voucher number	Family	Organ used	EtOH extract (g)
1	*Alhagi graecorum* Boiss[1]	Camel Thorn, Manna tree	FUPD-1	Papilionaceae	Leaves	18.5
2	*Amaranthus lividus* L.[1]	Pigweed	FUPD-2	Amaranthaceae	Herb	29.7
3	*Anastatica hierochuntica* L.[3]	Dinosaur plant, Mary's flower, Palestinian tumble weed.	FUPD-3	Brassicaceae	Herb	13.5
4	*Artemisia Absinthium* L.[3]	Absinthe wormwood, green ginger	FUPD-4	Asteraceae	Leaves, Flowers	19.8
5	*Aster squamatous* Sprengel[1]	Aster, Starwort.	FUPD-5	Asteraceae	Herb	12.2
6	*Beta vulgaris var.cicla* L.[2*]	Spinach beet	FUPD-6	Chenopodiaceae	Leaves	18.7
7	*Camellia sinensis* L. Kuntze[3*]	Green tea plant	FUPD-7	Theaceae	Leaves	30.5
8	*Cartagena ipecacuanha* Brot.[3]	Cartagena Ipecacuanha, Rio ipecac	FUPD-8	Rubiaceae	Root	109.3
9	*Chenopodium murale* L.[1]	Nettle leaf, Goose foot	FUPD-9	Chenopodiaceae	Herb	22.8
10	*Cichorium endivia* L.[2*]	Cultivated Endive.	FUPD-10	Asteraceae	Leaves	23.5
11	*Cichorium intybus* L.[3]	Chicory.	FUPD-11	Asteraceae	Roots, Leaves	36.8
12	*Cinnamomum cassia* (Nees and T.Nees) Farw.[3*]	Chinese Cassia, Chinese Cinnamon.	FUPD-12	Lauraceae	Bark	11.4
13	*Citrus reticulata* Blanco[2]	Mandarin orange, West African Cherry Orange	FUPD-13	Rutaceae	Leaves	32.7
14	*Conyza dioscoridis* L. Desf.[1]	Horseweed, Butterweed, Fleabane	FUPD-14	Asteraceae	Herb	14.1
15	*Corchorus olitorius* L.[2*]	Jew's Mallow	FUPD-15	Tiliaceae	Leaves	17.8
16	*Curcuma aromatica* Salisb.[3*]	Curcuma	FUPD-16	Zingiberacea	Rhizome	11.1

[1]Wild plants, [2]cultivated plants, [3]plants bought from herbal markets. *Edible vegetables and herbal teas consumed in Upper Egypt district.

Table 2: Selected medicinal plants from Upper Egypt for biological screening against leukemia K562 cell line

No.	Latin name	Common name	Voucher number	Family	Organ used	EtOH extract (g)
17	*Cymbopogon Proximus* Spreng.[3]	Halfa bar	FUPD-17	Poaceae	Leaves	8.6
18	*Cyperus alopecuroides* Rottbn.[1]	Foxtail flat sedge	FUPD-18	Cyperaceae	Leaves, Flowers	20
19	*Cyperus Rotundus* L.[1]	Nut grass, Tiririca	FUPD-19	Cyperaceae	Leaves, Flowers	19.1
20	*Daucus carota* L.[2]	Wild carrot, Queen Anne's lace	FUPD-20	Apiaceae	Leaves	23.7
21	*Desmostachia bipinnata* L. Stapf[1]	Halfa grass, Big cord grass, Salt reed-grass	FUPD-21	Poaceae	Herb	8.8
22	*Emblica officinalis* L. Kurz[3]	Amla, Indian Gooseberry	FUPD-22	Phyllanthaceae	Herb	43.4
23	*Eruca sativa* Mill.[2*]	Salad Rocket, Rucoli, Rugula	FUPD-23	Brassicaceae	Leaves	25.9
24	*Ficus carica* L.[2]	Common fig	FUPD-24	Moraceae	Leaves	12.2
25	*Glycyrrhiza glabra* L.[3*]	Liquorice	FUPD-25	Fabaceae	Roots, Rhizomes	14.1

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26	*Hibiscus sabdariffa* L.[3*]	Roselle, Karkadeh	FUPD-26	Malvaceae	Flowers calyx and epicalyx	45.4
27	*Hyphaene thebaica* L. Mart.[3*]	Doum palm, Gingerbread tree	FUPD-27	Arecaceae	Fruits	46.4
28	*Lawsonia inermis* L.[3]	Loose strife, Egyptian privet, Henna	FUPD-28	Lythraceae	Leaves	19.6
29	*Lupinus albus* L.[3*]	Lupine	FUPD-29	Fabaceae	Seeds	14.6
30	*Malva parviflora* L.[2*]	Cheese weed, Egyptian mallow, Marshmallow	FUPD-30	Malvaceae	Leaves	15
31	*Mentha longifolia* L. Huds.[1*]	Horsemint	FUPD-31	Lamiaceae	Herb	17.7

[1]Wild plants, [2]cultivated plants, [3]plants bought from herbal markets. *Edible vegetables and herbal teas consumed in Upper Egypt district.

Table 3: Selected medicinal plants from Upper Egypt for biological screening against leukemia K562 cell line

No.	Latin name	Common name	Voucher number	Family	Organ used	EtOH extract (g)
32	*Morus alba* L.[2]	White mulberry	FUPD-32	Moraceae	Leaves	15.6
33	*Opuntia ficus indica* L. Mill.[2]	Prickly pear	FUPD-33	Cactaceae	Leaves	16.3
34	*Origanum majorana* L.[3*]	Sweet marjoram	FUPD-34	Lamiaceae	Herb	22.7
35	*Peganum harmal* L.[3]	Harmal, Syrian rue	FUPD-35	Nitrariaceae	Seeds	12.2
36	*Phaseolus vulgaris* L.[2]	French bean	FUPD-36	Papilionaceae	Leaves	18.1
37	*Phragmites communis* (Cav.) Trin. ex Steud.[1]	Common reed	FUPD-37	Poaceae	Leaves, Flowers	8.1
38	*Pimpinella anisum* L.[3*]	Aniseed, Anise	FUPD-38	Apiaceae	Fruits	14.9
39	*Psidium guajava* L.[2]	Guava	FUPD-39	Myrtaceae	Leaves	10.1
40	*Punica granatum* L.[2]	Pomegranate	FUPD-40	Lythraceae	Fruit pericarp	31.9
41	*Quercus infectoria* gall[3]	Aleppo oak	FUPD-41	Fagaceae	Galls	85.6
42	*Ricinus communis* L.[1]	Castor	FUPD-42	Euphorbiaceae	Leaves	24.5
43	*Salix subserrata* Willd.[1]	Salix	FUPD-43	Salicaceae	Leaves	22.4
44	*Sesamum indicum* L.[2]	Sesame	FUPD-44	Pedaliaceae	Leaves	17.2
45	*Sesbania sesban* L. Merr.[1]	River hemp, Sesban	FUPD-45	Fabaceae	Leaves	76.5
46	*Sisymbrium irio* L.[1]	London rocket	FUPD-46	Brassicaceae	Herb	24.3
47	*Solenostemm aargel* (Delile) Hayne[3]	Argel	FUPD-47	Apocyanaceae	Leaves	31.4
48	*Spinacia oleracea* L.[2*]	Spinach	FUPD-48	Chenopodiaceae	Leaves	14.2
49	*Tamarindus indica* L.[3*]	Tamarind	FUPD-49	Fabaceae	Fruit, Seeds	60.6
50	*Tamarix nilotica* L.[1]	Nile tamarisk	FUPD-50	Tamaricaceae	Herb	17.2
51	*Thymus vulgaris* L.[2*]	Thyme	FUPD-51	Lamiaceae	Herb	14.9
52	*Tilia cordata* Mill.[3*]	Small leaved Lime	FUPD-52	Tiliaceae	Leaves, Flowers	11.8
53	*Trifolium alexandrinum* L.[2]	Egyptian Clover	FUPD-53	Fabaceae	Leaves	25.4

[1]Wild plants, [2]cultivated plants, [3]plants bought from herbal markets. *Edible vegetables and herbal teas consumed in Upper Egypt district.

Table 4: Selected medicinal plants from Upper Egypt for biological screening against leukemia K562 cell line

No.	Latin name	Common name	Voucher number	Family	Organ used	EtOH extract (g)
54	*Withania somnifera* L. Dunal.[1]	Ashwagandha, Indian Ginseng	FUPD-54	Solanaceae	Leaves	17.5
55	*Zingiber officinale* Roscoe[3*]	Ginger	FUPD-55	Zingiberaceae	Rhizome	12.9
56	*Ziziphusspina-christi* L. Desf.[2]	Christ's Thorn Jujube	FUPD-56	Rhamnaceae	Leaves	18.9
41	*Quercus infectoria* gall[3]	Aleppo oak	FUPD-41	Fagaceae	Galls	85.6
42	*Ricinus communis* L.[1]	Castor	FUPD-42	Euphorbiaceae	Leaves	24.5
43	*Salix subserrata* Willd.[1]	Salix	FUPD-43	Salicaceae	Leaves	22.4

44	*Sesamum indicum* L.[2]	Sesame	FUPD-44	Pedaliaceae	Leaves	17.2
45	*Sesbania sesban* L. Merr.[1]	River hemp, Sesban	FUPD-45	Fabaceae	Leaves	76.5
46	*Sisymbrium irio* L.[1]	London rocket	FUPD-46	Brassicaceae	Herb	24.3
47	*Solenostemm aargel* (Delile) Hayne[3]	Argel	FUPD-47	Apocyanaceae	Leaves	31.4
48	*Spinacia oleracea* L.[2*]	Spinach	FUPD-48	Chenopodiaceae	Leaves	14.2
49	*Tamarindus indica* L.[3*]	Tamarind	FUPD-49	Fabaceae	Fruit, Seeds	60.6
50	*Tamarix nilotica* L.[1]	Nile tamarisk	FUPD-50	Tamaricaceae	Herb	17.2
54	*Withania somnifera* L. Dunal.[1]	Ashwagandha Indian Ginseng	FUPD-54	Solanaceae	Leaves	17.5
55	*Zingiber officinale* Roscoe[3*]	Ginger	FUPD-55	Zingiberaceae	Rhizome	12.9
56	*Ziziphus spina-christi* L. Desf.[2]	Christ's Thorn Jujube	FUPD-56	Rhamnaceae	Leaves	18.9

[1]Wild plants, [2]cultivated plants, [3]plants bought from herbal markets. *Edible vegetables and herbal teas consumed in Upper Egypt district.

Preparation of plant extracts

The air-dried powder of each plant material (100 g) was extracted with 75% ethanol (500 mL×3). Each extract was filtered off and concentrated using a rotary evaporator (BUCH, rotavapor® R-300). Each residue was weighed and stored in a refrigerator at -4°C until used.

Trypan blue exclusion cytotoxicity assay using leukemia K562 cells

Human chronic leukemia K562 cells were purchased from the American Type Culture Collection, Rockville MD, USA. The cell line was grown in suspension culture at 37ºC in RPMI-1640 medium supplemented with 10% non-dialyzed fetal bovine serum (FBS), 2 mM L-glutamine, 100 units/mL of penicillin, 10 µg/ML of streptomycin, 2.5 µg/mL fungizone, 10% heat-activated fetal calf serum, and 2 mM glutamine. Cells were allowed to grow at 37º C in a humidified atmosphere of 5% CO_2 and 95% air to form a monolayer. At 60-70% confluence, cells were sub-cultured; first they were washed with phosphate-buffered saline (PBS), then trypsinized with 3 mL of 0.25% trypsin in 0.03% EDTA, then washed with fresh medium and seeded at 1 x 10^4 cells/well in a 96-well microplate. K562 cells were set up at 1 x 10^5 cells/well in Costar 24-well plates. The cells were allowed to grow undisturbed for 24 h before the addition of extracts. After 48 h incubation with extracts at 37ºC, viable cell counts were made by using the trypan blue exclusion method to assess cell viability. Taxol was used as a standard.[21-23] The extracts were tested at a concentration of 10 mg/mL and the percentage inhibitions were calculated.

Results and Discussion

The 75% ethanol extracts of fifty-six medicinal plants were screened for their antileukemic activity against the leukemia K562 cell line at a concentration of 10 mg/mL and the percentage of inhibition was calculated (Table 5, Figures 1 and 2). Fourteen plant extracts demonstrated more than 50% inhibition of the leukemia K562 cells. The percentages of inhibition include extracts from *Sesbania sesban* L. Merr leaves (100%), *Curcuma aromatica* Salisb. roots (90.2%), *Spinacia oleracea* L. leaves (88.9%), *Quercus infectoria* gall (87%), *Thymus vulgaris* L. leaves (85.2%), *Trifolium alexandrinum* L. leaves (78.2%), *Aster squamatous* Sprengel aerial parts (74%), *Withania somnifera* L. Dunal. Leaves (67.5%), *Ziziphusspina-christi* L. Desf. leaves (66.6%), *Emblica officinalis* L. Kurz aerial parts (65.9%), *Camellia sinensis* L. Kuntze leaves (56.5%), *Psidium guajava* L. leaves (54.2%), *Sisymbrium irio* L. aerial parts (52.5%), and *Morus alba* L. leaves (52.2%), compared to Taxol (90.7%) as shown in Table 5 and Figure 2.

Sesbania sesban, *Curcuma aromatica*, *Spinacia oleracea*, *Quercus infectoria* gall, and *Thymus vulgaris* were chosen as the top five active plants. There are no current reports about the cytotoxicity of *Sesbania sesban* against the K562 cell line. Phytochemical screening of the leaves of this plant showed the presence of steroids such as *β*-sitosterol and campesterol,[24] triterpenoids such as betulinic and oleanolic acids,[25,26] saponins of oleanolic acid,[27-30] flavonoids,[31,32] and coumarins.[33] However, the cytotoxic activity of *Curcuma aromatica* against K562 cells was previously described, and the activity of *Curcuma* extract was attributed to the twenty-five isolated curcuminoids, and the cytotoxicity of these compounds was evaluated against K562 cells as well.[34]

Although, the antileukemic activity of the whole extract of *Spinacia oleracea* leaves against the K562 cell line has been previously reported,[35,36] none of these studies described the detailed phytochemistry of the plant or its antileukemic metabolites. From the *Spinacia oleracea* L. leaves, several phytoconstituents such as flavonoids,[37-41] steroids,[42,43] triterpenoid saponin,[44] phenolic acids,[45,46] carotenoids,[47] and glycolipids,[48] have been isolated. It also contains the major vitamins, such as vitamins C, A, and E, as well as minerals such as iron, calcium, potassium, manganese, and zinc.[49] The ethanol extract of *Quercus infectoria* galls (*Andricus sternlichti* and *Andricus moreae*) showed cytotoxic effects with IC_{50} values of 21.52 and 39.14 µg/mL, respectively, on the K562 cell line.[50] The gall is known to be rich in gallic acid, which is reported as an antileukemic compound that inhibits the proliferation of K562 cells through inhibition of BCR/ABL kinase and NF-κB inactivation.[51] The antileukemic activity of the total extract of *Thymus vulgaris* leaves or its volatile oil against the K562 cell line was previously described.[52,53] The cytotoxic activity of Thymol, the major constituent of this plant, was also estimated against the K562 cells. Thymol exhibited antiproliferative activity (IC_{50} = 400-500 µM) by significantly reducing the level of DNA damage induced in K562 cells by the strong oxidant, H_2O_2.[54]

Conclusion

The study showed that *Sesbania sesban* leaves, *Curcuma aromatica* roots, *Spinacia oleracea* leaves, *Quercus infectoria* gall, and *Thymus vulgaris* leaves were the top five active plants against leukemia K562 cells. The findings of the study reveal that the flora of Upper Egypt can be a source of structurally diverse metabolites that could lead to the development of numerous drugs to treat CML.

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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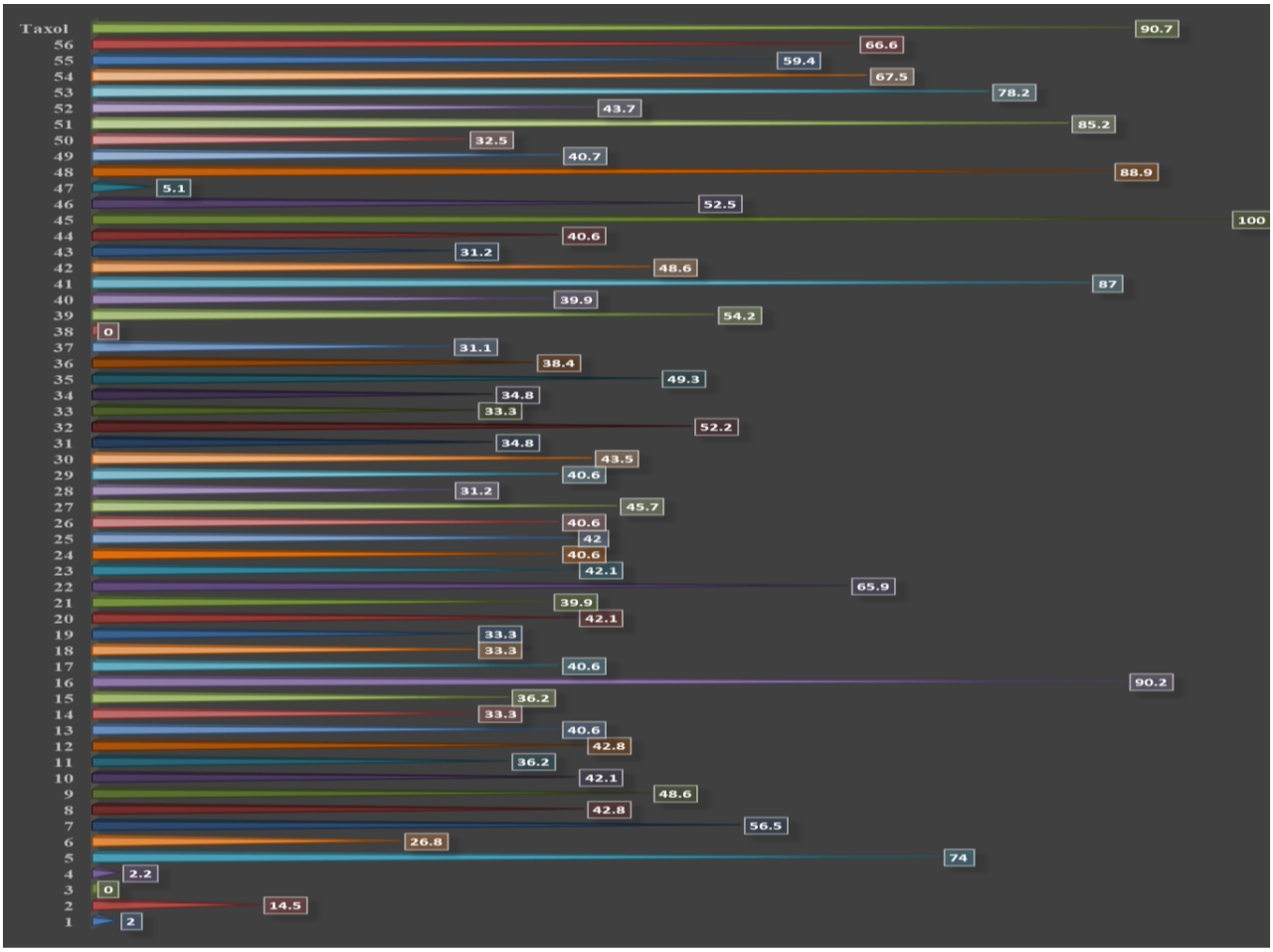

Figure 1: Percentage inhibition of fifty-six plant extracts from Upper Egypt against K562 cell line

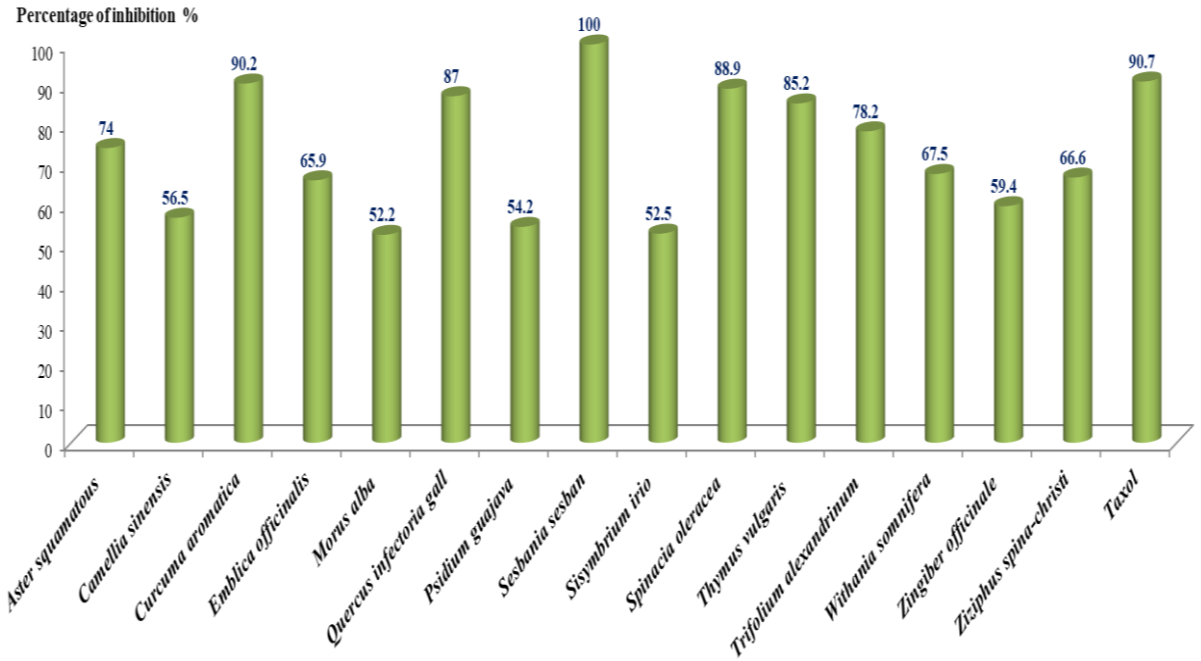

Figure 2: Percentage of inhibition of most active selected extracts against K562 cell line at a conc. of 10 mg/mL

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Table 5: Percentage of inhibition (%) of 56 selected plant extracts against K562 cell line at a conc. of 10 mg/mL

No.	Plant name	Inhibition (%)	No.	Plant name	Inhibition (%)	No.	Plant name	Inhibition (%)
1	*Alhagi graecorum* Boiss	2.0	20	*Daucus carota* L.	42.1	39	*Psidium guajava* L.	54.2
2	*Amaranthus lividus* L.	14.5	21	*Desmostachia bipinnata* L. Stapf	39.9	40	*Punica granatum* L.	39.9
3	*Anastaticahier ochuntica* L.	0.0	22	*Emblica officinalis* L. Kurz	65.9	41	*Quercus infectoria* gall	87.0
4	*Artemisia Absinthium* L.	2.2	23	*Eruca sativa* Mill.	42.1	42	*Ricinus communis* L.	48.6
5	*Aster squamatous* Sprengel	74.0	24	*Ficus carica* L.	40.6	43	*Salix subserrata* Willd.	31.2
6	*Beta vulgaris* var. cicla L.	26.8	25	*Glycyrrhiza glabra* L.	42.0	44	*Sesamum indicum* L.	40.6
7	*Camellia sinensis* L. Kuntze	56.5	26	*Hibiscus sabdariffa* L.	40.6	45	*Sesbania sesban* L. Merr	100.0
8	*Cartagena ipecacuanha* Brot.	42.8	27	*Hyphaene thebaica* L. Mart.	45.7	46	*Sisymbrium irio* L.	52.5
9	*Chenopodium murale* L.	48.6	28	*Lawsonia inermis* L.	31.2	47	*Solenostemma argel* (Delile) Hayne	5.1
10	*Cichorium endivia* L.	42.1	29	*Lupinus termis* L.	40.6	48	*Spinacia oleracea* L.	88.9
11	*Cichorium intybus* L.	36.2	30	*Malva parviflora* L.	43.5	49	*Tamarindus indica* L.	40.7
12	*Cinnamomum cassia* (Nees & T.Nees) Farw.	42.8	31	*Mentha longifolia* L. Huds.	34.8	50	*Tamarix nilotica* L.	32.5
13	*Citrus reticulate* Blanco	40.6	32	*Morus alba* L.	52.2	51	*Thymus vulgaris* L.	85.2
14	*Conyza dioscoridis* L. Desf.	33.3	33	*Opuntia ficus indica* L. Mill.	33.3	52	*Tilia cordata* Mill.	43.7
15	*Corchorus olitorius* L.	36.2	34	*Origanum majorana* L.	34.8	53	*Trifolium alexandrinum* L.	78.2
16	*Curcuma aromatica* Salisb.	90.2	35	*Peganum harmal* L.	49.3	54	*Withania somnifera* L. Dunal.	67.5
17	*Cymbopogon Proximus* Spreng.	40.6	36	*Phaseolus vulgaris* L.	38.4	55	*Zingiber officinale* Roscoe	59.4
18	*Cyperus alopecuroides* Rottb.	33.3	37	*Phragmites communis* (Cav.) Trin. ex Steud.	31.1	56	*Ziziphus spina-christi* L. Desf.	66.6
19	*Cyperus Rotundus* L.	33.3	38	*Pimpinella anisum* L.	0.0	57	Taxol	90.7

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