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Cytotoxic effect of the ethanolic extract of *Calotropis procera* flowers on human cancer cell lines

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ABSTRACT

Conventional cancer treatments are often associated with adverse effects, prompting researchers to investigate alternative anticancer agents deriving from natural sources, including traditional medicinal plants. One such plant, *Calotropis procera* L., is rich in phytochemicals with broad pharmacological potential. This study has evaluated the cytotoxic effects of its flower's extract on two cancer cell lines – human osteosarcoma (MG63) and human glioblastoma (A172) – using normal human dermal fibroblasts (neonatal; HdFn) as a control. The MTT assay was employed in order to assess cytotoxicity and apoptosis induction. Our results demonstrate a dose-dependent inhibition of cell growth. The most effective concentrations of the *Calotropis procera* flower extract were 400, 200, and 100 µg/mL, with MG63 cells showing greater sensitivity than A172 cells. No cytotoxicity was observed in HdFn cells. The IC_{50} values were 213.7 µg/mL for MG63, 282.66 µg/mL for A172, and 844.08 µg/mL for HdFn. The selective cytotoxicity of the extract is attributed to the presence of potent phytochemicals that induce apoptosis in cancer cells without harming healthy cells. These findings suggest that the flower extract of *Calotropis procera* may serve as a promising and safe anticancer agent.

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1. Introduction

Cancer remains a major global health challenge, significantly impacting human populations. There is a need for novel therapies that can treat and prevent this life-threatening disease without

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Table 1. Viability of MG63, A172, and HdFn cells following a 24-h treatment with varying concentrations of a Calotropis procera flower extract. Identical superscript letters indicate no statistically significant difference ($p>0.05$) between the groups, while different superscript letters denote significant differences ($p<0.05$).

Cell viability (%; mean ± standard deviation)					
	***Calotropis procera* flower (ethanolic) extract** (μg/mL)				
Cell lines	400	200	100	50	25
MG63	37.80 ± 1.88 ^{c}	43.17 ± 1.14 ^{c}	54.89 ± 1.09 ^{c}	64.04 ± 2.15 ^{c}	77.08 ± 2.23 ^{c}
A172	42.97 ± 4.46 ^{c}	51.65 ± 2.98 ^{c}	59.68 ± 1.54 ^{c}	73.95 ± 1.72 ^{b}	86.72 ± 1.30 ^{b}
HdFn	74.65 ± 2.57 ^{a}	86.34 ± 1.53 ^{a}	93.59 ± 2.10 ^{a}	94.17 ± 1.57 ^{a}	95.21 ± 0.82 ^{a}

causing adverse effects. The plant kingdom offers a vast reservoir of naturally-occurring secondary metabolites, many of which are under investigation for their anticancer properties and have contributed to the development of new drugs[1]. *Calotropis procera* L., a wild-growing perennial flowering plant of the Apocynaceae family, is considered a rich source of diverse phytochemicals. Each part of the plant contains bioactive compounds with distinct therapeutic significance, including triterpenoids, anthocyanins, flavonoids, cardiac glycosides, cardenolides, α-amyrin, β-amyrin, lupeol, β-sitosterol, flavanols, resin, the potent bacteriolytic enzyme calactin, and the non-toxic proteolytic enzyme calotropin. The plant has demonstrated a wide range of pharmacological activities, including antimicrobial, anthelmintic, anti-inflammatory, analgesic, antipyretic, anticancer, antiangiogenic, immunomodulatory, antidiabetic, cardiovascular, hypolipidaemic, gastroprotective, hepatoprotective, nephroprotective, antioxidant, anticonvulsant, and wound-healing effects[2]. Despite its broad therapeutic potential, the anticancer efficacy of *Calotropis procera* flowers has not been previously explored. This study investigates the cytotoxic activity of the flower extract of *Calotropis procera* on MG63 (human osteosarcoma) and A172 (human glioblastoma) cells.

2. Methodology

2.1. Preparation of a raw powder of *Calotropis procera* flowers

Fresh, fully bloomed, and healthy flowers of *Calotropis procera* were collected in March 2024 from Hillah, Iraq. The plant material was identified and authenticated by Dr Shaimaa Mohi (Department of Biology, College of Science, University of Babylon). The flowers were cleaned so as to remove dust and were air-dried in the shade at room temperature. Once dried, they were ground into a fine powder using an electric mill, and stored in a sterile, airtight container for later use[3].

2.2. Preparation of a *Calotropis procera* flower (ethanolic) extract

The powdered flowers were made into an extract using 70% ethanol in a 1:10 (w/v) ratio. The mixture was shaken at 100 rpm for 2 h, followed by incubation in a water bath at 40°C for an additional 2 h. The extract was then filtered through filter paper, and the filtrate was dried in an oven at 45°C. The resulting solid extract was ground using an electric grinder, sterilized under UV light for 20 min, and stored in a sterile, dark, and tightly sealed vial[4].

2.3. MTT assay

The MTT assay is a colorimetric method used in order to assess cell viability by measuring the reduction of yellow tetrazolium bromide to dark purple formazan crystals by the mitochondrial succinate dehydrogenase in metabolically active cells. The intensity of the colour, measured at 570 nm, correlates with the number of viable cells. The assay was performed using an MTT Kit (Intron Biotech, Korea), which includes MTT reagent and a solubilization

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solution. A total of 100 µL of cell suspension (MG63, A172, or HdFn) was seeded into flat-bottomed microculture plate wells and incubated at 37°C in a 5% CO_2 atmosphere for 24 h. After incubation, 100 µL of the *Calotropis procera* flower extract at concentrations of 25, 50, 100, 200, or 400 µg/mL was added to the wells. Absorbance was measured using an ELISA reader at 570 nm. The cell viability (%) was calculated using the formula:

cell viability (%) = (absorbance of treated cells / absorbance of control) × 100

The control consisted of growth medium with MTT dye only, representing 100% viability. IC_{50} values were calculated by using the GraphPad Prism software (multiple parameter analysis).

2.4. Statical analysis

Data were analysed using SPSS version 23. One-way analysis of variance (ANOVA), followed by Duncan's multiple range test, was used in order to determine significant differences between groups. A *p*-value lower than 0.05 was considered as statistically significant.

3. Results and Discussion

The cytotoxic effects of the *Calotropis procera* flower extract were evaluated against two human cancer cell lines, namely MG63 (osteosarcoma) and A172 (glioblastoma), using the MTT assay. This colorimetric method is based on the reduction of pale yellow tetrazolium salt (MTT) by mitochondrial dehydrogenase enzymes in viable cells, resulting in the formation of dark purple formazan crystals. These crystals accumulate within metabolically active cells, and their quantity is directly proportional to cell viability[5].

To assess cytotoxicity, various concentrations (25, 50, 100, 200, and 400 µg/mL) of the 70% ethanolic extract of the *Calotropis procera* flowers were applied to MG63, A172, and normal human dermal fibroblast (HdFn) cells. After 24 h of incubation, a dose-dependent reduction in cell viability was observed, as shown in Table 1. The most pronounced effects were seen at concentrations of 100, 200, and 400 µg/mL, with MG63 cells exhibiting greater sensitivity than A172 cells. Specifically, the MG63 cell viability decreased to 77.08%, 64.04%, 54.89%, 43.17%, and 37.80% across the five assessed concentrations, with an IC_{50} value of 213.7 µg/mL. On the other hand, A172 cell viability was recorded at 86.72%, 73.95%, 59.68%, 51.65%, and 42.97%, with an IC_{50} of 282.66 µg/mL. In contrast, HdFn cells maintained higher viability levels of 95.21%, 94.17%, 93.59%, 86.34%, and 74.65%, with an IC_{50} of 844.08 µg/mL.

The extract demonstrated selective cytotoxicity, significantly reducing the viability of cancer cells while sparing normal cells (Table 1). MG63 cells were more susceptible to the extract than A172 cells. This selective cytotoxicity is likely attributed to the presence of bioactive phytochemicals in the *Calotropis procera* flower extract, such as cinnamic acid derivatives, rosmarinic acid, and β-amyrin, which have been shown to reduce cancer cell viability without affecting normal cells[6–8]. Supporting studies have reported similar findings, attributing the cytotoxic effects of *Calotropis procera* flower extract to its high polyphenolic content[6,9] and its ability to elevate intracellular reactive oxygen species, thereby inducing cytotoxicity, apoptosis, and autophagy in cancer cells such as MCF-7[10].

4. Conclusion

The ethanolic extract of *Calotropis procera* flowers exhibits potent cytotoxic activity against cancer cells (MG63 and A172), while sparing normal human dermal fibroblasts. This selective action suggests that the *Calotropis procera* flower extract might serve as a promising and safe anticancer agent, with minimal adverse effects on healthy human tissues.

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Conflicts of interest

None exist.

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References

1. Greenwell M., Rahman P.K. Medicinal plants: their use in anticancer treatment. *Int. J. Pharm. Sci. Res.* 6(10), 4103–4112, 2015. DOI: 10.13040/IJPSR.0975-8232.6(10).4103-12
2. Brunda M.A., Bindhu M.A., Ashok Kumar B.S., Mouna A., Subhashini B. Ethnomedicinal and cytotoxic activities of *Calotropis procera* – a review. *Asian J. Pharm. Res. Dev.* 12(3), 125–128, 2024. DOI: 10.22270/ajprd.v11i3.1408
3. Al Ghezi N.A.S., Al-Mossawi A.E.B.H.J., Al-Rikabi A.K.J. Antioxidants activity of date seed extraction of some date varieties. *Med. Leg. Update* 20(1), 922–928, 2020. DOI: 10.37506/v20/i1/2020/mlu/194714
4. Ekpenyong C.E., Akpan E.E., Udoh N.S. Phytochemistry and toxicity studies of *Telfairia occidentalis* aqueous leaves extract on liver biochemical indices in Wistar rats. *Am. J. Med. Med. Sci.* 2(5), 103–110, 2012. DOI: 10.5923/j.ajmms.20120205.03
5. Hassan F., El-Hiti G.A., Abd-Allateef M., Yousif E. Cytotoxicity anticancer activities of anastrozole against breast, liver hepatocellular, and prostate cancer cells. *Saudi Med. J.* 38(4), 359–365, 2017. DOI: 10.15537/smj.2017.4.17061
6. Al-Taweel A.M., Perveen S., Fawzy G.A., Rehman A.U., Khan A., Mehmood R., *et al.* Evaluation of antiulcer and cytotoxic potential of the leaf, flower, and fruit extracts of *Calotropis procera* and isolation of a new lignan glycoside. *Evid. Based Complement. Alternat. Med.* 2017, 8086791, 2017. DOI: 10.1155/2017/8086791
7. Pal A., Tapadar P., Pal R. Exploring the molecular mechanism of cinnamic acid-mediated cytotoxicity in triple negative MDA-MB-231 breast cancer cells. *Anticancer Agents Med. Chem.* 21(9), 1141–1150, 2021. DOI: 10.2174/1871520620666200807222248
8. Maiyo F., Moodley R., Singh M. Phytochemistry, cytotoxicity and apoptosis studies of β-sitosterol-3-O-glucoside and β-amyrin from *Prunus africana*. *Afr. J. Tradit. Complement. Altern. Med.* 13(4), 105–112, 2016. DOI: 10.21010/ajtcam.v13i4.15
9. Smit H.F., Woerdenbag H.J., Singh R.H., Meulenbeld G.J., Labadie R.P., Zwaving J.H. Ayurvedic herbal drugs with possible cytostatic activity. *J. Ethnopharmacol.* 47(2), 75–84, 1995. DOI: 10.1016/0378-8741(95)01255-c
10. Al-Qahtani M.A.M., Farah M.A., Abou-Tarboush F.M., Al-Anazi K.M., Al-Harbi N.O., Ali M.A., *et al.* Anticancer effects of *Calotropis procera* latex extract in MCF-7 breast cancer cells. *Pharmacogn. Mag.* 16(71), 550–556, 2020. DOI: 10.4103/pm.pm_156_20

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