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Phytochemical profiling, antioxidant potential, and hematological safety of Apple of Sodom extract: An In vitro study

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Abstract

Apple of Sodom (Calotropis procera) is a medicinal plant rich in bioactive compounds that is attracting increasing attention from researchers for its potential role in reducing oxidative stress. This study was conducted to evaluate the antioxidant activity and hematological safety of Apple of Sodom extract. Chromatographic-mass spectrometry (GC-MS) analysis was used to identify the active chemical compounds in the extract, and the antioxidant activity was assessed using the DPPH free radical scavenging assay. The extract's biosafety was also assessed using a hemolysis assay. Gas Chromatography-Mass Spectroscopy analysis revealed six major compounds with relative abundances ≥1%, including Amrinone at 67.64%, followed by Vasicinone at 20.49%, as well as other compounds such as Harmine and Deoxypeganine. DPPH assay results indicated that the extract possesses concentration-dependent antioxidant activity. At a concentration of 0.12 mg/mL, the free radical inhibition increased from 63.52% to 90.19%, and at a concentration of 1 mg/mL, it increased to ~ 100 %. Based on these results, the IC_{50} value for antioxidant activity was 0.09 ± 0.01 mg/mL, indicating high efficacy of the extract at relatively low concentrations. Regarding the hemolysis results, the alcoholic extract showed no hemolysis at any concentration, with a 0% hemolysis rate compared to the positive control, reflecting its high hematological safety. The results of this study indicate that the Apple of Sodom plant extract possesses antioxidant activity along with good biosafety, supporting its potential for future pharmaceutical uses.

Keywords: Apple of Sodom; GC MS; DPPH; Hemolysis; ROS

1. Introduction

Oxidative stress is a major contributing factor to the development of many diseases, such as inflammatory disorders, heart disease, and some types of cancer, especially when it results from the accumulation of free radicals and reactive oxygen species (Jomova et al., 2023). The side effects of synthetic antioxidants have increased concern and led to attention being directed towards their natural sources, including medicinal plants, as they are rich in active compounds capable of reducing oxidative stress effectively and safely (Xu et al., 2017). The Sodom apple (Calotropis procera), belonging to the Apocynaceae family, is a wild, flowering, evergreen shrub widespread in desert and semi-desert environments. It has been traditionally used in folk medicine to treat inflammation, diarrhea, malaria, fever, diabetes, and many skin diseases for a long period of human beings (Shafiq et al., 2024). This has led researchers to examine its chemical makeup and possible biological effects. Studies have shown that apple of sodom plant contains a wide range of active compounds, including alkaloids, cardiac glycosides, and phenolic compounds, which are linked to antioxidant and anti-inflammatory activities (Yaniv and Koltai et al., 2018; Ali, 2016). Plant extracts rich in alkaloids, even in the absence of flavonoids, can exhibit remarkable antioxidant activity by promoting cellular stability or interfering with reactive oxygen species. Some Calotropis procera extracts have shown free radical inhibition rates at concentrations below 1 mg/mL exceeding 70%, with IC_{50} values below 0.1 mg/mL, indicating antioxidant efficacy (Brand-Williams et al., 1995; Kedare and Singh, 2011; Yaniv and Koltai, 2018).

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Hematological safety, along with bioavailability, is a crucial factor in evaluating the potential applications of plant extracts, as some active compounds may damage red blood cell membranes, thus limiting their therapeutic use (Akintimehin et al., 2021). The hemolysis test is commonly used as a main method for checking biocompatibility. Hemolysis rates below 5% show acceptable hemodynamic safety (von Petersdorff-Campen and Daners, 2022).

Therefore, our current study aimed to chemically characterize Calotropis procera extract, evaluate its antioxidant activity, calculate its IC_{50} value, and assess its hematologic safety.

2. Materials and Methods

2.1. Preparation of the Plant Extract

The aerial parts of Apple of Sodom were collected from the Al-Shabakah region near the Saudi Arabian border (260 km from the center of Najaf Governorate, Iraq). They were then cleaned and dried at room temperature, away from direct sunlight, and ground into a fine powder. The active compounds were extracted using maceration with 70% ethanol, as described by Harborne (1998). The plant powder was macerated in the solvent for 72 hours with periodic stirring. The extract was then filtered using filter paper and concentrated using a rotary evaporator under reduced pressure to obtain the crude extract, which was stored at 4°C until use.

2.2. Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

GC-MS analysis was performed according to the steps described by Adams (2007) to identify chemical compounds in the plant extract using a gas chromatography-mass spectrometer equipped with a suitable capillary column. The sample was injected after dissolution in a volatile solvent, and a step-up temperature program was used in the instrument's furnace (at 60 °C with a 120-second hold, then the temperature was increased at an average rate of 5 °C/min until reaching 280 °C, with a final 10-minute hold). By comparing the mass spectra with approved standard spectral libraries (NIST library), compounds were identified and matched. Only compounds with acceptable match quality and relative abundance ≥1% were included in the final analysis.

2.3. Antioxidant Activity Assessment Using the DPPH Test

The antioxidant activity of the alcoholic extract was assessed using the DPPH free radical scavenging assay. Following the steps described by Brand-Williams et al. (1995), different concentrations of the extract (0.12, 0.25, 0.5, and 1 mg/ml) were prepared. A specific volume of each extract was then mixed with freshly prepared DPPH solution, and the samples were incubated for 30 minutes in the dark at room temperature for an appropriate duration. Subsequently, the absorbance was measured using UV-Vis spectroscopy at a wavelength of 517 nm. The percentage of free radical inhibition (%) was calculated based on the difference between the absorbance of the control and the sample.

2.4. Calculating the IC_{50} Value of Antioxidant Activity

The IC_{50} value, which is the concentration required to achieve 50% inhibition of free radicals, was calculated by plotting the relationship between the inhibition rates obtained from the DPPH test and the concentrations of the plant extract. Appropriate regression analysis was then performed to accurately determine the IC_{50} value (Kedare and Singh, 201)

2.5. Hemolysis Assay

The hematologic safety of the plant extract was evaluated using a hemolysis assay for red blood cells, following the steps described by Yang et al. (2015). Fresh blood samples were collected, and red blood cells were isolated by centrifugation and washed with physiological saline. The cells were exposed to different concentrations of the plant extract, with distilled water serving as the positive control and physiological saline as the negative control. After an appropriate incubation period, the optical absorbance of the supernatant was measured, and the percentage of hemolysis was calculated compared to the positive control.

2.6. Statistical Analysis

All experiments were conducted with three independent replicates, and results were presented as mean ± standard deviation (Mean ± SD). Appropriate statistical analysis was used to assess the significance of differences, and values were considered statistically significant at the $p < 0.05$ level (Motulsky, 2014).

3. Results and discussion

3.1. Phytochemical analysis using mass spectrometry

The data of table (1) and Figure (1) of the GC-MS analysis of the alcoholic extract of Apple of Sodom revealed the identification of six major compounds with relative abundances ≥1%. The results indicated that the vast majority of these compounds are relatively non-volatile alkaloids, with Amrinone being the most abundant at 67.64%, followed by Vasicine at 20.49%. Other compounds were detected in smaller quantities, including n-Hexadecanoic acid, harmine, deoxygenize, and 4-Amino-2-ethyl-3-methylquinoline. Although Amrinone is primarily known as a synthetic compound, similar quinolinone derivatives have been reported in plant-associated matrices, warranting further confirmatory analyses.

These alkaloids, in general, possess multiple biological activities, including the ability to scavenge free radicals and interfere with reactive oxygen species. Therefore, it is likely that the observed antioxidant activity of the extract is due to a synergistic effect between these alkaloid compounds, with the possibility of a supporting effect from other compounds in this alcoholic extract. This is consistent with what (Chen et al., 2022) indicated and what (Salman et al., 2026) found.

Table 1 GC–MS identified compounds in the plant extract

Compound	R.T. (min)	Area (%)	CAS No.
Amrinone	22.78	67.64	060719-84-8
Vasicine	24.02	20.49	000486-64-6
n-Hexadecenoic acid	23.00	3.37	000057-10-3
Deoxygenize	21.68	1.09	000495-59-0
Hermine	25.98	1.22	000442-51-3
4-Amino-2-ethyl-3-methylquinoline	23.12	1.05	065197-40-2

Only compounds with relative abundance ≥1% and acceptable library matching were reported.

3.2. Antioxidant activity using DPPH assay

The results in Table (2) related to the DPPH test indicate that the alcoholic extract possesses antioxidant activity depending on the concentration. As the extract concentration increased from 63.52% at 0.12 mg/mL, the percentage of free radical inhibition increased, showing a clear dose-dependent increase to 90.19% at the highest concentration of 1 mg/mL. This trend confirms the extract's effectiveness in neutralizing free radicals and reflects a direct relationship between the extract's concentration and the inhibition rate (Figure 1). The value of IC_{50} for antioxidant activity was calculated based on the data in Table 2 and the corresponding graph in Figure 1, resulting in a value of 0.09 ± 0.01 mg/mL. This reflects the extract's high activity at relatively low concentrations. The gradual increase in the free radical inhibition rate with increasing extract concentration indicates the ability of the active compounds to react with free radicals via a dose-dependent mechanism. Furthermore, the relatively low IC_{50} value is an important quantitative indicator of the extract's antioxidant efficacy, reflecting the need for a limited concentration to achieve significant free radical inhibition. This aligns with the findings of (Aini and Tilaqza, 2025).

Table 2 Antioxidant activity of plant extract using DPPH assay

Sample name	Concentration	Scavenging %
1	0.12 mg/ml	63.522
2	0.25 mg/ml	77.704
3	0.5 mg/ml	79.543
4	1 mg/ml	90.192
	control	

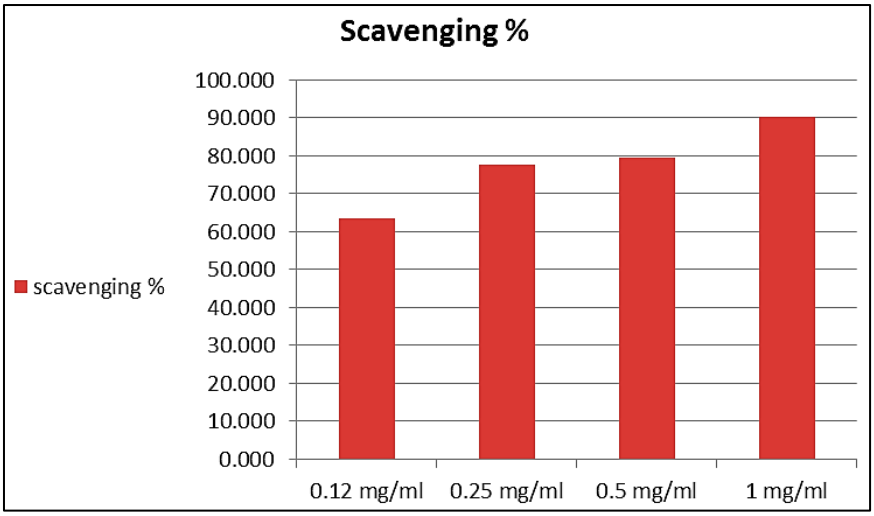

Figure 1 Concentration vs % scavenging (DPPH)

3.3. Hematological safety assessment of plant extract

The data in Table 3 show that the alcoholic extract of Apple of Sodom, at all tested concentrations (0.25, 0.5, and 1 mg/mL), did not cause hemolysis. In all treatments, the hemolysis rate was 0%, with uptake values of 0.244, 0.323, and 0.357, respectively. The positive control exhibited 100% complete hemolysis with a high uptake value of 2.392, while the negative control showed no hemolysis (0%) with an uptake value of 0.379. These results indicate that the plant extract has high hemologic safety within the limits of the tested concentrations and within the safe limit of the hemolysis test (<5%). These results are consistent with what Júnior et al. (2019) found, that the absence of hemolysis or its remaining within the minimum limits is a reliable indicator of hemologic safety and the possibility of using plant extracts in pharmaceutical and biological applications.

Table 3 Effect of plant extract on red blood cell breakdown

Concentration (mg/ml)	Test result	Hemolysis %
1	0.357	0
0.5	0.323	0
0.25	0.244	0
POSITIVE	2.392	100
NEGATIVE	0.379	0

4. Conclusion

The study results indicated that the alcoholic extract is rich in bioactive compounds and possesses high concentration-dependent antioxidant activity with a low IC_{50} value, in addition to exhibiting complete hemolysis without any recorded hemolysis. These findings confirm the plant's potential as a natural and safe source of antioxidants, with promising possibilities for future pharmaceutical and nutritional applications.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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