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Phytochemical Characterization and Evaluation of the Antioxidant and Cytotoxic Activities of *Cressa cretica* L.

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Abstract : Halophytic *Cressa cretica* L. (Convolvulaceae) is long recognized for possessing medicinal activity in arid and saline habitats, but comprehensive analysis of its phytochemical content and bioactivity is not enough. The primary objectives of this study was to identify the secondary metabolites of *C. cretica* and assess its antioxidant and cytotoxic activities. Qualitative phytochemical examining of the methanolic extract revealed the presence of alkaloids, flavonoids, phenols, glycosides, terpenes, tannins, saponins, and steroids, and phenolics and flavonoid constituents were abundant. The extract showed that it could scavenge DPPH free radicals in a concentration-dependent way, with 63.8 ± 1.79% inhibition at 500 mg/mL and an IC_{50} value of 40.51 mg/mL, showing moderate antioxidant activity relative to vitamin C (IC_{50} = 7.15 mg/mL). Cytotoxicity in the MTT assay expressed selective, dose-dependent inhibition of human hepatocellular (HepG-2) and colorectal (HCT-116) carcinoma cell growth with IC_{50} values of 43.37 µg/mL and 31.65 µg/mL, correspondingly, without expressing much cytotoxicity to normal WI-38 cells (IC_{50} > 100 µg/mL). These biological activities are attributed mainly to the plant's abundant content of phenolic and flavonoid compounds. The findings highlight *C. cretica* as a valuable source of anticancer and antioxidant metabolites with potential pharmaceutical and nutraceutical industry implications, warranting its traditional medicinal application.

keywords: *Cressa cretica,* halophyte, phytochemical screening, cytotoxicity, DPPH assay.

1. Introduction

In recent years, wild plants that grow naturally in salty or arid habitats have attracted much scientific attention. Their ability to survive under extreme stress is often correlated to complex metabolic systems that generate a variety of defensive compounds. In coastal area plants such as *Cressa cretica* L. (Convolvulaceae), a small halophytic shrub broadly found across the deserts and semi-deserts of the North Africa, Middle East, and South Asia. Traditionally, it has been used to treat fever and jaundice as a general health tonic, and in some regions it also serves as livestock fodder in saline lands [1].

Earlier chemical studies have shown that *C. cretica* is rich in several groups of bioactive compounds such as flavonoids (e.g., quercetin and kaempferol derivatives), phenolic acids (including chlorogenic acid), sterols (β-sitosterol), tannins, alkaloids, and coumarins [2]. Other analyses, including those by Suthar and Solanki [3], confirmed the presence of alkaloids, phenols, tannins, and saponins in its aerial parts. Operating GC–MS and FTIR, Omran et al. [4] detected nearly 30 additional constituents, such as phenols, alkyl halides, aldehydes, and carboxylic acids. Finally, these findings indicate that a chemically rich species that importance further researching for its antioxidant and cytotoxic activities.

The correlation between a plant's phenolic and flavonoid content and its antioxidant activity is well known [5]. For *Cressa cretica*, Singh et al. [6] showed that the methanol extract of the aerial parts could significantly lower liver lipid peroxidation and restore the antioxidant enzymes SOD, CAT, and GPx in rats treated with CCl_4. These results point to clear antioxidant and hepatoprotective effects. In a similar study, El-Alfy et al. [7] examined

different extracts of *C. cretica* from Egypt and found that both in-vitro and *in-vivo* tests supported its antioxidant and liver-protective activity. Overall, these findings make *C. cretica* a strong candidate as a natural source of antioxidants.

Apart from its antioxidant role, some early reports have also hinted at cytotoxic potential. Fawzi et al. [8] found that the ethyl-acetate and ethanolic fractions of Iraqi *C. cretica* showed cytotoxic effects on human cancer cell lines, and the flavonoid astragalin (kaempferol-3-O-glucoside) was isolated through HPLC. The review by Jaafar et al. [1] also mentioned in-vivo cytotoxic effects of ethyl-acetate extracts on mouse bone marrow and spleen cells [9]. These observations highlight the need for deeper studies on its anticancer potential.

Despite the available data, comprehensive studies that link the chemistry of *C. cretica* with its biological effects are still rare. Therefore, this work aims to (i) assess its main secondary metabolites using chromatographic and spectrometric tools, (ii) test its antioxidant properties through standard in-vitro assays such as DPPH, and (iii) evaluate its cytotoxic activity against HepG-2 and HCT-116 cancer cell lines to explore possible connections between composition and activity.

2. Materials and Methods

2.1. Plant collection

Fresh aerial parts of *Cressa cretica* L. were collected during the flowering season (2024) from the coastal desert along the deltaic coast of Egypt. The plant was identified and authenticated according to the taxonomic descriptions of Boulos [10], and a voucher specimen (Mans.003003003004) was dropped in the Herbarium of the Department of Botany, Faculty of Science, Mansoura University, Egypt. To get rid of dust and dirt, the samples were properly cleaned, let to air-dry in the shade at room temperature for two weeks, and then ground into a fine powder with an electric mill. The powdered material was saved in airtight containers at room temperature until subsequent phytochemical and biological analyses.

2.2. Qualitative phytochemical screening

Preliminary phytochemical investigation of the powdered aerial parts of *Cressa cretica* was performed to detect major classes of secondary metabolites following the standard methods of Harborne [11] and Trease and Evans [12], with minor modifications. The reagents of Mayer's and Dragendorff were used to test for the presence of alkaloids indicated by the precipitation of creamy white or reddish-brown colored precipitates. The use of magnesium turnings along with concentrated hydrochloric acid proved to be a test for flavonoids based on the Shinoda test with a resultant pinkish or red color appearance. Phenols and tannins were detected by the ferric chloride test, producing blue-black or greenish hues, respectively.

Saponins were determined with frothing test in which sustained foam formation indicated their presence. Glycosides were identified with Keller–Killiani test that showed a reddish-brown ring at the interface, a hallmark sign of cardiac glycosides. The Salkowski test was used to detect terpenoids as indicated by reddish-brown color at the interface, while steroids were confirmed with Liebermann–Burchard test that resulted in the appearance of a greenish-blue color. Anthraquinones were identified with Borntrager test in which appearance of pink to red color after treatment with ammonia indicated their presence. All these assays were performed in triplicate, and the results recorded qualitatively as present or absent.

2.3. Antioxidant Activity

With minimal changes, Blois's [13] procedure for 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenger evaluation was used to determine the antioxidant content of methanolic extract from *Cressa cretica* One milliliter of the extract at different concentrations (50, 100, 200, 300, 400, and 500 mg/mL) was combined with one milliliter of 0.1 mM DPPH prepared in methanol. The resultant reaction mix was well agitated and incubated for thirty minutes at room temperature in the dark. The reduction of absorbance at 517 nm was then determined using a UV-Vis spectrophotometer with methanol as control blank. Ascorbic acid was used as standard antioxidant in analysis. The scavenger activity of DPPH radical was determined from the following expression:

% Inhibition (RSA) = [(A of control −A of sample)/A of control]×100

where Aof control is the absorbance of the control (DPPH solution without extract) and A of sample is the absorbance in the presence of the extract. The IC_{50} value, representing the extract concentration required to achieve 50% inhibition of DPPH radicals, was obtained from the dose–response curve. All measurements were carried out in triplicate, and the results were expressed as mean ± standard deviation (SD).

2.4. Cytotoxic activity

Herein, A1 is used to represent absorbance in the presence of the extract, while A0 is that of the control, namely the DPPH solution without the extract. The IC_{50} value for the concentration of the extract needed to give 50% inhibition of DPPH radicals was determined from the examination of the dose-response curve. Each was repeated three times as a measurement and its results presented as mean ± standard deviation (SD).

The MTT colorimetric assay reported by Bondock et al. [14] and Terblanche et al. [15] was used as a method to determine cytotoxicity. This technique is based on the fact that mitochondrial dehydrogenases in viable cells reduce yellow MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide] to form purple formazan. The cells seeded at 1×10^4 cells per well in 96-well plates were exposed to different sample concentrations for 24 hours. The formazan crystals then dissolved in 100 µL DMSO after incubating with MTT solution (5 mg/mL, 20 µL) for 4 hours. The absorbance was then determined at 570 nm using a microplate reader (EXL 800, USA). The IC_{50} values were estimated using nonlinear regression with Origin 8.0 software (OriginLab Corp., USA). The equation was used to determine the reduction in percentage inhibition as well as percent relative cell viability:

$$\% \text{ Inhibition} = (A_{\text{of control}} - A_{\text{of sample}})/(A_{\text{of control}}) \times 100$$

3. Results and Discussion

3.1. Qualitative phytochemical screening

The qualitative phytochemical screening of *Cressa cretica* discovered the occurrence of a diverse array of bioactive secondary metabolites, comprising alkaloids, flavonoids, phenols, tannins, steroids, glycosides, anthraquinones, terpenes, and saponins, albeit in varying intensities. Among these, flavonoids, phenols, glycosides, and anthraquinones were strongly detected (+++), indicating that these compounds may constitute the major phytochemical groups in this species. Tannins and steroids were moderately present (++), while alkaloids, saponins, and terpenes were detected in relatively lower concentrations (+) .

The phytochemical screening of *C. cretica* (Table 1) showed the occurrence of several key secondary metabolites, involving alkaloids, flavonoids, phenols, glycosides, and terpenes, with moderate to high abundance. These results agree with earlier studies reporting similar constituents in *C. cretica* collected from arid and saline habitats [3, 16]. The pronounced presence of flavonoids and phenols is remarkably important, as these mixtures are well known for their antioxidant and free-radical-scavenging properties [2].

The detection of alkaloids, glycosides, and terpenes further supports the plant's broad pharmacological potential, including antimicrobial and cytotoxic effects [17]. Saponins, tannins, and steroids were present in lower amounts, consistent with previous reports showing moderate concentrations of these compounds in halophytes [10]. The absence of anthraquinones also aligns with the chemical profile typically observed for *C. cretica* [18].

Overall, the predominance of phenolic and flavonoid compounds suggests that *C. cretica* is a promising basis of natural antioxidants, supporting its traditional medicinal employs and highlighting its adaptive metabolic strategy in saline desert environments..

Table 1. Qualitative phytochemical evaluation of a few untamed plants gathered from the desert coastline.

Screening test	Cressa cretica
Alkaloids	++
Flavonoids	++
Phenols	++
Saponins	+
Tannins	+
Steroids	+
Glycosides	++
Anthraquinones	-
Terpenes	++

"+++" = high concentration; "++" = moderate; "+" = low; "±" = trace; "–" = absent.

3.2. Antioxidant Activity

The DPPH radical scavenging results (Table 2) demonstrate that the methanolic extract of *Cressa cretica* exhibits a clear, concentration-dependent antioxidant activity. The scavenging percentage increased from 7.96 ± 0.32 % at 50 mg/mL to 63.8 ± 1.79 % at 500 mg/mL, with an IC_{50} of 40.51 mg/mL, indicating moderate antioxidant potential. In comparison, vitamin C, used as a positive control, showed markedly higher activity (IC_{50} = 7.15 mg/mL), consistent with its well-established potency as a reference antioxidant [13].

The gradual increase in antioxidant activity with higher concentrations indicates that the extract can donate hydrogen atoms or electrons to neutralize free radicals. This activity is mainly attributed to its polyphenols and flavonoids, which are well known for their redox potential and ability to scavenge reactive oxygen species [16, 2]. Similar to other halophytic species adapted to oxidative stress, *C. cretica* shows a moderate IC_{50} value (< 50 mg/mL), reflecting a considerable level of natural antioxidant potential [18].

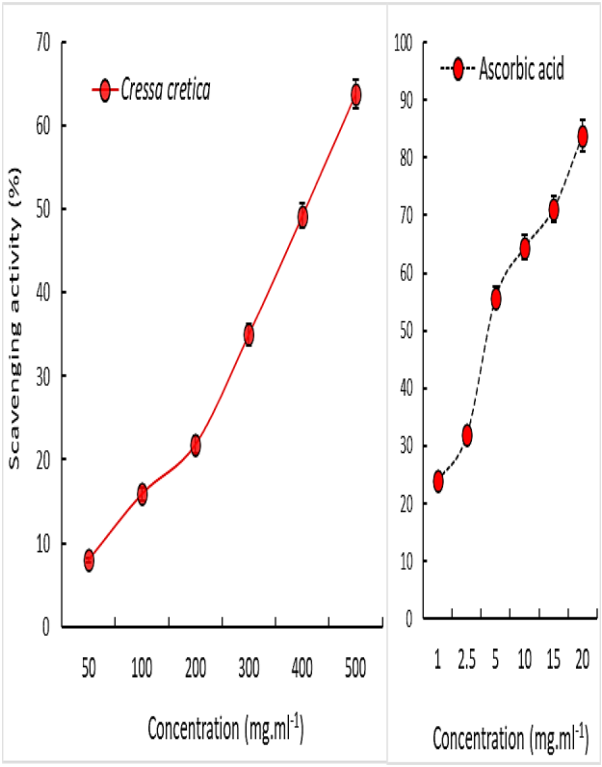

Figure 1. Scavenging activity percentage of DPPH and the IC_{50} values by MeOH extract of Cressa cretica and vitamin C as standard.

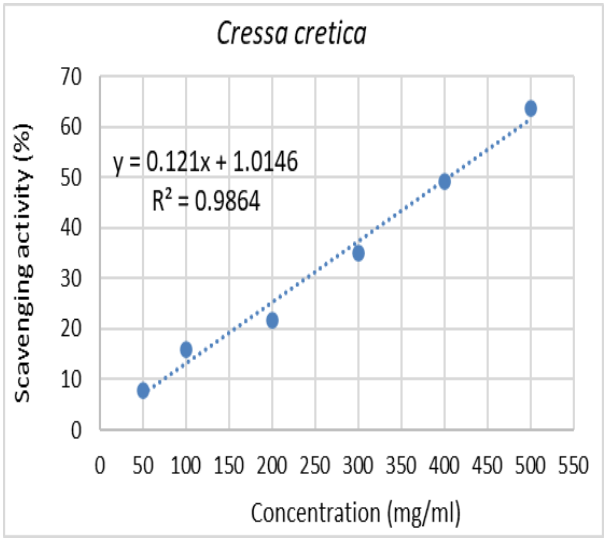

Figure 2. Scavenging activity standard curves shown against the concentrations of the tested samples.

Previous studies support this observation. Suthar and Solanki (2021) reported that *C. cretica* methanolic extracts exhibited significant DPPH radical inhibition, ascribed to its excessive total phenolic (22.95 mg GAE/g) and flavonoid (10.18 mg CE/g) contents. These compounds act synergistically to stabilize free radicals through resonance, metal chelation, and hydrogen donation mechanisms [17]. The moderate antioxidant capacity observed in this study thus correlates well with the phytochemical profile obtained earlier (Table 1), which indicated a rich presence of phenols, flavonoids, and glycosides.

Moreover, the antioxidant potential of *C. cretica* can be associated with its ecological adaptation. As a halophytic species thriving in saline desert environments, it accumulates protective metabolites such as phenolics and terpenes to mitigate salt-induced oxidative stress [16]. This ecological pressure likely enhances its antioxidant machinery, making it a promising source of natural antioxidants for pharmaceutical and nutraceutical applications.

Although the extract's scavenging activity was lower than that of pure vitamin C, its effectiveness at higher concentrations confirms that *C. cretica* possesses bioactive compounds capable of neutralizing free radicals. Further purification and chromatographic identification of its active phenolic and flavonoid components are required to elucidate the compounds primarily accountable for their antioxidant potential.

Table 2. DPPH scavenging activity % and IC_{50} values using *Cressa cretica* MeOH extract and standard vitamin C.

Treatment	Concentration (mg/ml)	Scavenging activity (%)	IC_{50} (mg/ml)
Cressa cretica	50	7.96 ± 0.32	**40.51**
	100	15.88 ± 0.77	
	200	21.8 ± 0.98	
	300	35.6 ± 1.25	
	400	49.2 ± 1.46	
	500	63.8 ± 1.79	
Vitamin C	1	24.01±0.77	**7.15**
	2.5	31.90±1.03	
	5	55.76±1.80	
	10	64.43±2.07	
	15	71.10±2.29	
	20	83.78±2.61	

3.3. Cytotoxic activity

Figure 3 presents the results of the cytotoxic test for the methanolic extract of *Cressa cretica* and Doxorubicin. The standard drug Doxorubicin was highly active, with IC_{50} values of 6.05 µg/mL for HePG-2 and 11.57 µg/mL for HCT-116. Its IC_{50} value was above 100 µg/mL for the normal WI-38 cells, showing that it is strong but selective in its action [19].

Similarly, *C. cretica* extract demonstrated a dose-dependent cytotoxic response, with the highest inhibition (60.47% and 69.3%) observed at 100 µg/mL for HePG-2 and HCT-116, respectively. The calculated IC_{50} values were 43.37 µg/mL (HePG-2) and 31.65 µg/mL (HCT-116), indicating moderate cytotoxic potential compared with Doxorubicin, yet showing minimal toxicity toward normal WI-38 cells (IC_{50} >100 µg/mL). The selective activity suggests that *C. cretica* contains bioactive compounds capable of targeting cancer cells while sparing normal ones.

The cytotoxicity of *C. cretica* may be attributed to its flavonoids, phenolics, glycosides, and terpenoids, which have been reported to induce apoptosis, DNA fragmentation, and oxidative stress in tumor cells [20-22]. Comparable findings were reported by Al-Madhagy et al. (2018), who observed significant anticancer activity of *C. cretica* extracts against hepatic and colon carcinoma cells. The findings thus point to *C. cretica* as a potentially useful natural source of cytotoxic drugs, indicating the need for more bioassay-guided separation of its active ingredients.

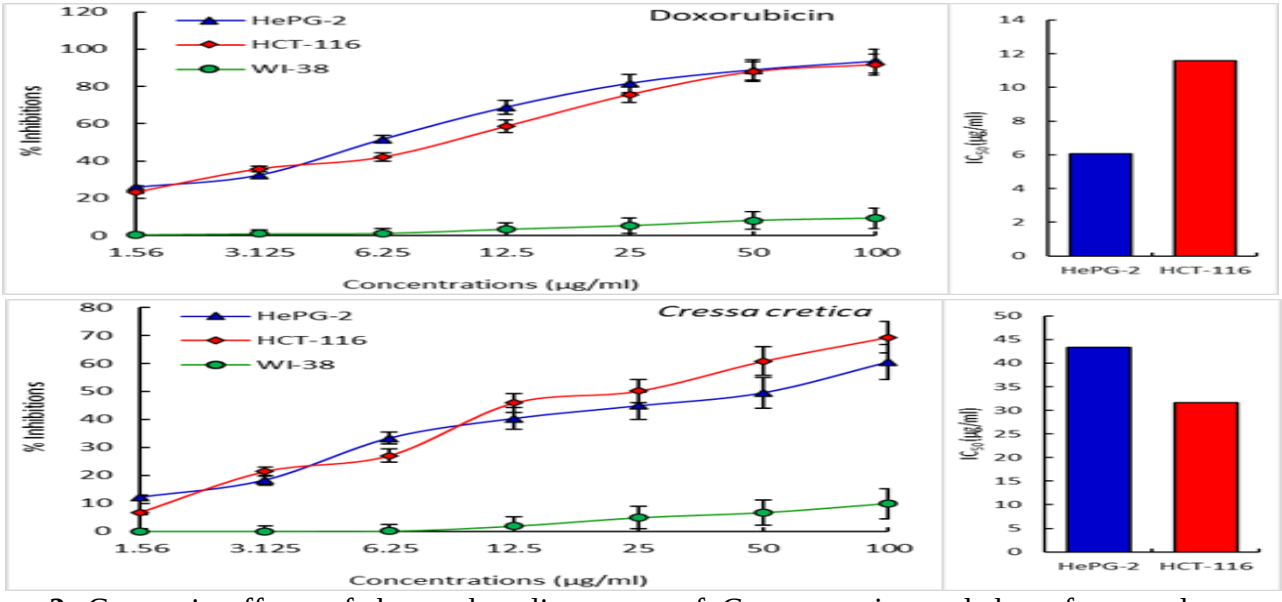

Figure 3. Cytotoxic effects of the methanolic extract of *Cressa cretica* and the reference drug Doxorubicin versus HePG-2, HCT-116, and WI-38 cell lines

4. Conclusion

The findings of this study show that *Cressa cretica* ., a halophyte that thrives in desert environments, contains a wide variety of phytochemicals with notable biological properties. The methanolic extract was rich in phenols, flavonoids, glycosides, terpenes, alkaloids, and tannins, all of which may contribute to its antioxidant and cytotoxic effects. The extract showed moderate free radical scavenging activity that increased with concentration, reflecting its potential as a natural antioxidant. It also demonstrated selective cytotoxicity toward HepG-2 and HCT-116 cancer cells, with little influence on normal cells.

Altogether, these results support the long-standing medicinal use of *C. cretica* and point to its possible pharmaceutical application as a natural source of bioactive compounds. Further isolation and in vivo testing of the active components are recommended to understand their mechanism of action.

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